VOLUME 26 NUMBER 4

PLANT PHYSIOLOGY

OCTOBER, 1951

CONTENTS

Sulphur Nutrition of Cotton. David R. Eccles and Frank M. Eaton, Texas Agricultural Experiment Station, College Station, Texas ... 689

Further Theoretical Considerations of Modes of Expression and Factors Possibly Concerned in the Movement of Materials through a Two-Phased Solution System. T. C. Broyer, University of California, Berkeley, California ... 695

Biochemical Composition of Cotton Leaves and Their Chemical Defoliation as Affected by Environment. Vernon L. Hall, University of Arkansas, Fayetteville, Arkansas ... 697

Difference in Activity between 2,4-Dichlorophenoxyacetic Acid and Other Auxins, and Its Significance in Herbicidal Action. J. van Overbeek, René Blondeau, and Vesta Horner, Shell Oil Company, Modesto, California ... 707

Enzymatic Conversion of Indole Acetaldehyde and Naphthalene Acetaldehyde to Auxins. Poul Larsen, University of Chicago, Chicago, Illinois ... 707

Physiological and Biochemical Changes Accompanying Pollination in Orchid Flowers. II. Respiration, Catalase Activity, and Chemical Constituents. Tsung-Hsun T. Hsiang, Morehouse College, Atlanta, Georgia ... 708

Causes of Injury to Plants Resulting from Flooding of the Soil. Paul J. Kramer, Duke University, Durham, North Carolina ... 722

The Nutrient-Element Balance of the Tomato and Its Susceptibility to Phytophthora infestans as Affected by Two Levels of Zinc. Cyril B. Smith, Pennsylvania State College, State College, Pennsylvania 727

The Effects of the Form of Nitrogen and the Amount of Base Supply on the Organic Acids of Tung Leaves. Seymour G. Gilbert, Cornelius B. Shear, and Clara M. Grove, University Station, Gainesville, Florida ... 739

Investigations of Ascorbic Acid Dehydrogenase of Peas (Pisum sativum) and Its Distribution in the Developing Plant. M. Yamaguchi and M. A. Joslyn, University of California, Berkeley, California ... 747

(Continued on Cover Page Four)

PUBLISHED QUARTERLY

BY

THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS

AT 111 E. CHESTNUT ST., LANCASTER, PA.

Entered as second-class matter March 25, 1926, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

Accepted for mailing at the special rate of postage provided for in paragraph 4, section 412. Authorized April 9, 1923.

PLANT PHYSIOLOGY

A QUARTERLY JOURNAL
DEVOTED TO ALL PHASES OF PLANT PHYSIOLOGY

Established 1926

WALTER F. LOEHWING, *Editor-in-chief*, 1954
State University of Iowa

ASSOCIATE EDITORS

C. H. WADLEIGH, 1955
U.S.D.A. Plant Industry Station
Beltsville, Maryland

M. W. PARKER, 1954
U.S.D.A. Plant Industry Station
Beltsville, Maryland

J. B. BIALE, 1953
University of California
Los Angeles, California

IRENE H. STUCKEY, 1952
Rhode Island Agricultural
Experiment Station

H. B. VICKERY, 1956
Connecticut Agricultural
Experiment Station

F. P. CULLINAN, 1955
U.S.D.A. Plant Industry Station
Beltsville, Maryland

C. S. FRENCH, 1953
Carnegie Institution
Stanford, California

F. C. GUSTAFSON, 1952
University of Michigan
Ann Arbor, Michigan

ROBERT M. MUIR, *Secretary of the Editorial Board*, 1956
State University of Iowa

H. A. BOYTHWICK, 1954
U.S.D.A. Plant Industry Station
Beltsville, Maryland

P. J. KRAMER, 1953
Duke University
Durham, North Carolina

E. J. KRAUS, 1952
Oregon State College
Corvallis, Oregon

K. C. HAMNER, 1954
University of California
Los Angeles, California

R. EMERSON, 1953
University of Illinois
Urbana, Illinois

J. P. BENNETT, 1952
University of California
Berkeley, California

The pages of this journal are open to papers of merit dealing with any phase of Plant Physiology. Membership in the Society is not required of contributors, but non-members are required to pay a fee of $20.00 for each paper printed. Manuscripts must be typewritten, and must have the approval of the Editorial Board. Papers must conform to the limitations established by the Society at the St. Louis meeting, 1935. Proofs should be returned with necessary corrections, and reprints ordered when the proof is returned. Reprints can be furnished with or without covers at cost of production. Correspondence with reference to editorial matters should be addressed to the Editor, DR. WALTER F. LOEHWING, Department of Botany, State University of Iowa, Iowa City, Iowa.

Subscription price, $10.00 a year. Single copies, $3.00 post free. Members of the American Society of Plant Physiologists receive the journal in connection with dues paid to the Society. Missing numbers will be supplied if lost in the mail, provided that written notice is received within one month of the date of issue; otherwise they will be furnished at the single copy rate, as long as they are available.

Correspondence concerning membership and its privileges, dues, change-of-address, etc., should be addressed to the Executive Secretary-Treasurer, DR. J. FISHER STANFIELD, Miami University, Oxford, Ohio. Business correspondence regarding subscriptions, back numbers, change-of-address of subscribers, etc., may be addressed to PLANT PHYSIOLOGY, 111 E. Chestnut St., Lancaster, Pa., or to the Executive Secretary-Treasurer at the address above. All matters pertaining to nominations, elections, programs, and meetings of the society should be addressed to the Secretary, DR. A. S. CRAFTS, University of California, Davis, California.

PLANT PHYSIOLOGY

VOLUME 26 OCTOBER, 1951 NUMBER 4

SULPHUR NUTRITION OF COTTON[1]

DAVID R. ERGLE AND FRANK M. EATON

(WITH ONE FIGURE)

Received March 30, 1951

The role of sulphur in the metabolism of the tomato, and some other plants, was the object of a critical study by NIGHTINGALE *et al.* (**14**) in 1932. This work was followed by a succession of three papers by S. V. EATON (**8, 9, 10**) dealing respectively with soybeans, sunflowers, and black mustard. In each of the foregoing studies, attention was given to the distribution of sulphur between selected plant parts and to the effects of sulphur deficiency on carbohydrate and nitrogen metabolism. A large measure of emphasis was placed by the authors on proteolysis. BEESON (**2**) has shown by his analyses that the total sulphur in plants tends to approach or may exceed in concentration that of total phosphorus. COOPER and MITCHELL (**5**) found that cotton plants cultured under various conditions contained an average of 0.28% of sulphur and 0.24% of phosphorus on the dry weight basis. In a few instances (**4, 12, 18**), cotton has been shown to respond under field conditions to sulphur fertilization. The fact that attention to the sulphur relations in the nutrition of cotton has been somewhat limited finds one explanation in that sulphur is an ingredient in superphosphate as well as in some of the nitrogen and potash fertilizers.

The present study of the sulphur nutrition of the cotton plant has tended to follow lines of inquiry similar to those followed by Nightingale *et al.* and Eaton. In the discussion of the cotton data, differences and similarities are pointed to among the foregoing five plants. The data on cotton include: (1) distribution and forms of sulphur in cotton plants grown on a fertile soil; (2) the effects of sulphur deficiency on growth and mineral accumulation, and (3) the forms and concentrations of sulphur, carbohydrates and nitrogen in plants grown in sand cultures with two and with five levels of sulphate supply. In each experiment, analyses were made before and after the plants had flowered. Consideration is given to the effects of sulphur supply on fruitfulness.

[1] Approved as Technical Paper no. 1411 of the Texas Agricultural Experiment Station.

Analytical methods

Carbohydrates: The methods used in determining the various fractions have been outlined elsewhere (**7**).

Nitrogen: Total nitrogen (a) was determined by the Kjeldahl method after preliminary reduction with reduced iron powder. Soluble nitrogen (b) was similarly determined and includes all nitrogenous compounds extractable from the dried tissue with water at 80° C. Soluble organic nitrogen (c) was determined upon an aliquot of the extract obtained in (b) omitting the nitrate-reduction step. Nitrate-N was calculated as the difference between (b) and (c). Alpha-amino nitrogen was determined upon an aliquot of (b) using the Van Slyke method. Protein nitrogen was calculated as the difference between (a) and (b).

Sulphur fractions: Duplicate one and two gram tissue samples were Soxhlet-extracted with 80% ethanol. Sulphur determinations were made on both the extracts and alcohol-insoluble residues. Sulphate sulphur in one of the duplicate extracts was determined in the usual manner after precipitation as $BaSO_4$ and the evaporation of the alcohol. The total sulphur content of the second extract was determined by the official (**1**) magnesium nitrate method. The difference between total and sulphate sulphur was designated soluble organic sulphur. One of the alcohol-insoluble residues was refluxed with 2.5% HCl for two hours, and sulphate sulphur in the resulting extract again determined as $BaSO_4$. Total sulphur was determined on the other alcohol-insoluble residues, as before, and the difference between total and sulphate sulphur was taken as a measure of the protein sulphur. Sulphate was calculated as the sum of the alcohol-soluble and -insoluble sulphates. Total sulphur represents the sum of the three sulphur fractions. When fractionation of sulphur was limited to total organic and sulphate sulphur, the former was calculated as the difference between total and sulphate sulphur.

Other constituents: Calcium, magnesium, and phosphorus were determined (the latter colorimetrically) by official (**1**) methods. The potassium determination followed the Wilcox (**15**) gravimetric procedure. The iron determination was by the Walker thiocyanate procedure as given by Yoe (**17**).

Experimental procedure

Distribution and forms of sulphur accumulated in plants on soil

The Stoneville 2B plants, providing the data for table I, were planted April 19, 1949, and grown outdoors in three-gallon jars of manured Houston Black Clay. In the preflower stage (three weeks in advance of the first flower), the leaves contained 87% of the total sulphur in the plants. Fifteen days after flowering, the dry weight per plant had increased 4.7 fold but the total sulphur per plant had increased only 2.3 fold. At this time the leaves, buds, and bolls contained 88% of the total sulphur in the plants. The leafy

TABLE I

CONCENTRATION OF SULPHUR FRACTIONS IN VARIOUS PARTS OF COTTON PLANTS GROWN ON FERTILE SOIL. DRY WEIGHT PER PLANT AND PERCENTAGES ON DRY WEIGHT

Plant parts	Dry weight	Sulphate S	Soluble org. S	Protein S	Total S
	gms.	%	%	%	%
Preflower, 43 days:					
Leaves	6.5	0.80	0.06	0.17	1.03
Stems and petioles	3.3	.15	.02	.06	.23
Root	2.0	.04	.03	.03	.10
Entire plant	11.8	.49	.05	.12	.66
Early fruiting, 79 days:					
Leaves	17.4	.55	.06	.14	.75
Stems and petioles	16.3	.05	.02	.03	.10
Root	11.9	.03	.01	.04	.08
Reproductive	9.8	.14	.03	.08	.25
Entire plant	55.4	.22	.02	.08	.32
Mature seed:					
Kernels	....	.02	.04	.31	.37

bracts of the buds and bolls constitute a substantial part of the weight of these organs but only in the instance of protein S was the concentration of any sulphur fraction as high as in the leaves. In the mature seed kernels, protein sulphur constituted 84% of the total sulphur. In the vegetative tissues this percentage was characteristically much lower. At both the first and second samplings, table I, sulphate S was the dominant form. Between the two samplings, there were no striking changes in the proportions of sulphate, soluble organic, or protein sulphur.

Plants in sand cultures supplied with minus S and plus S solutions

This planting of Stoneville 2B cotton was made in washed quartz sand in 30 tall four gallon stone jars in the greenhouse on March 14, 1949. After thinning to two seedlings per jar, half of the jars were supplied with millimolar concentrations of salts as follows: 5 $Ca(NO_3)_2$, 5 KNO_3, 2 $MgSO_4$, 1 KH_2PO_4 (the foregoing is Hoagland's solution), and 1 NaCl. The other jars received a like solution except that $MgCl_2$ was substituted for the $MgSO_4$. Both lots received 5 p.p.m. of boron, 0.5 p.p.m. of manganese, 0.05 p.p.m. of zinc and 0.01 p.p.m. of copper. Iron was supplied as magnetite mixed with the sand. De-ionized water and C.P. salts were used. The manufacturer's analyses indicated that the non-sulphate salts added 0.1 p.p.m. of SO_4 to the nutrient solutions.

New solutions were added always in amounts sufficient to produce drainage and in equal quantities to both the plus and minus S plants. Toward the end of the experiments, the nutrient-solution additions amounted to two liters per day. Only occasionally, on days of high transpiration, were the nutrient solutions supplemented by additions of de-ionized water.

One plant was removed from each jar for analyses on April 20, 36 days after planting, and the second one was taken on May 18, 64 days after planting. The dry weight per plant of the minus S plants increased from one gram at the first sampling to nine grams at the second sampling. The plus S plants increased from six grams to 46 grams. There was little proportionate difference in the gain with age between the plus and minus plants. Plant heights in the minus and plus groups were respectively 19 and 34 cm. at the first sampling and 31 and 59 at the second; *i.e.*, the SO_4 deficiency in this experiment reduced plant height by about half.

At both the first and second samplings, all minus S leaves were greenish yellow whereas all plus S leaves were green. In the minus S plants, there was no evident gradation of color between young and old leaves. The cotyledons of the minus S plants dropped between the first and second sampling and the oldest true leaves began to show deterioration. Leaves of corresponding age were much smaller on the minus S than on the plus S plants. The chemical data from this experiment are presented in table II.

Carbohydrates: At 36 days, remarkably little reducing and sucrose sugars were found in the tops of the minus S plants. At 64 days, no titratable sugars were found in the minus S leaves. The stems and petioles contained only one fifth as much sugar as was found in the plus S stems and petioles. On the other hand, the concentrations of starch and hemicellulose were reduced only moderately by sulphur deficiency at the first sampling and little or none at the second sampling.

Sulphur: In their various parts, the minus S plants contained only a quarter to an eighth as high percentage of total sulphur as the plus S plants.

Nitrogen: The minus S plant parts accumulated in the order of twice as high concentrations of nitrate and soluble organic nitrogen as did the plus S plants; the only exception being nitrate in the roots. On the other hand the plus S leaves contained almost twice as high concentrations of protein nitrogen as did the minus S leaves. This increase in protein N was not shown by the stems and roots.

Mineral elements: The minus S leaves accumulated nearly equal extra percentages of phosphorus and magnesium, but these accumulation effects were slight in the other tissues. There was some extra concentration of calcium in all minus S tissues. Potassium and iron concentrations were unaffected.

Effects through five levels of SO_4 supply

This experiment was conducted in the same manner as the foregoing one except that $MgCl_2$ was replaced with $MgSO_4$ in successive steps to produce five culture solutions containing 0.1, 1.0, 10, 50, and 200 p.p.m. of SO_4 ion. The planting was made in the greenhouse on March 17, 1950, and consisted of six jars for each SO_4 level.

Symptoms: At the time of the first sampling (the 35th day after planting) there was a step by step increase in the intensity of the green color of

TABLE II

DRY WEIGHT AND SULPHUR, CARBOHYDRATE, NITROGEN AND MINERAL ELEMENTS IN COTTON PLANTS AT 36 AND 64 DAYS WITH AND WITHOUT ADDED SULPHATE. DATA EXPRESSED AS PERCENTAGE OF DRY WEIGHT

Age and plant part	Dry weight (gm.)		Carbohydrates							
			Reducing sugars		Sucrose		Starch		Hemicellulose	
	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S
Preflower, 36 days:										
Entire tops	1.0	5.8	0.07	1.13	0.08	0.84	3.77	5.47	6.36	8.36
Early fruiting, 64 days:										
Leaf blades*	4.3	21.3	0.00	1.16	.00	1.44	8.70	8.86	7.45	7.28
Stems, petioles	3.1	18.6	.45	2.63	.29	2.43	9.90	11.68	14.18	14.58
Roots	1.6	5.8	2.36	2.13	3.04	3.84	17.26	14.59	12.74	13.55

	Sulphur (total)		Nitrogen							
			Nitrate		Solu. org.		Protein		Total	
	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S
Preflower, 36 days:										
Entire tops	0.11	0.86	1.44	0.63	1.51	0.67	1.23	2.38	4.18	3.68
Early fruiting, 64 days:										
Leaf blades*	.12	.91	0.17	0.07	1.83	0.59	1.55	2.65	3.55	3.31
Stems, petioles	.04	.17	.20	.14	1.45	0.39	1.58	1.28	3.23	1.81
Roots	.02	.12	.09	.16	.67	.29	0.41	0.42	1.17	.87

	Mineral Elements									
	Phosphorus		Potassium		Calcium		Magnesium		Iron	
	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S	Minus S	Plus S
Preflower, 36 days:										
Entire tops	0.37	0.35	4.06	3.83	4.47	3.61	0.88	0.79	....	....
Early fruiting, 64 days:										
Leaf blades*	0.56	.26	3.63	3.34	4.00	2.77	.58	.28	0.026	0.023
Stems, petioles	.24	.17	3.30	3.37	1.39	.91	.23	.18	....	....

*Samples included all leaves.

the leaves with increasing SO_4 supply. In the 0.1 and 1.0 p.p.m. levels, yellowing appeared within the first two weeks after planting. At 35 days, the plants supplied with 200 p.p.m. of SO_4 were greener than those supplied with 50 p.p.m. By 65 days, the color of the 50 and 200 p.p.m. SO_4 leaves was equal. At 65 days, the cotyledons of the 0.1 and 1.0 p.p.m. plants had abscised, and the oldest leaves were showing some deterioration.

Growth: Corresponding with the increasing intensity of chlorophyll color, step by step increases are shown at 35 days (table III) in both the fresh weight of tops and plant height (fig. 1) through the full series of sulphate supply levels. At 65 days, the plants in 50 and 200 p.p.m. of SO_4 had approximately equal weights. The height of plants was reduced more by sulphur deficiency in this experiment than in the previous one; at 65 days, the height of plants with 0.1 p.p.m. of sulphate was only one quarter

of the height of the 200 p.p.m. plants. The differentiation of main-stalk nodes was reduced to one half by sulphate deficiency. At the 0.1 SO_4 level, there were no fruiting or vegetative branches over 10 cm. long. Fifty parts per million of SO_4 gave as good growth and fruiting at 65 days (fig. 1) as did 200 p.p.m. Through the five levels of SO_4 supply there were respectively 0.5, 3.2, 15.8, 23.4, and 22.5 developing bolls per plant. As shown in table III sulphate supply did not influence relative fruitfulness, *i.e.*, there was no effect on the partition of growth between vegetative and fruiting activities. Similar relations have been shown elsewhere in the instance of nitrate supply.

Carbohydrates: Neither the entire tops at 35 days nor the leaf blades and main stalks at 65 days of the plants in either the 0.1 or 1.0 p.p.m. sul-

TABLE III

GROWTH AND FRUITING OF COTTON PLANTS AS INFLUENCED BY SULPHATE SUPPLY. AVERAGE VALUES PER PLANT

Treatment SO_4	Fresh tops	Height	Main-stalk nodes	Branches*		Relative fruitfulness**
				Fruiting	Vegetative	
p.p.m.	*gm.*	*cm.*	*number*	*number*	*number*	
			35 days—preflowering stage			
0.1	8.3	16	6.5	....	....	....
1	13.2	22	6.7	....	....	....
10	28.1	35	8.2	....	....	....
50	34.5	38	8.8	....	....	....
200	45.5	43	9.3	....	....	....
			65 days—early fruiting stage			
0.1	13	24	9.7	0.0	0.0	
1	50	41	12.0	0.2	0.0	4.0
10	237	86	15.6	5.2	0.0	3.8
50	350	100	17.8	8.2	1.2	4.0
200	345	103	18.0	8.3	0.8	3.8

*Length of 10 cm. or more.
**Number of developing bolls per 100 grams of fresh stems and leaves.

phate levels (table IV) contained titratable amounts of either reducing or sucrose sugars. Also, in general accord with the previous experiment, the percentage of starch was reduced moderately in entire plants by 0.1 p.p.m. of sulphate at 35 days and in the leaf blades at 65 days but not in the main stalks. The percentage of hemicellulose was essentially unaffected by the level of SO_4 supply.

Nitrogen: The percentage of total nitrogen was always greater when the SO_4 supply limited growth. The high accumulations of NO_3 and soluble organic nitrogen found at low SO_4 levels gave way to an accumulation of protein nitrogen at the higher SO_4 levels. Only in the instance of the woody stems did the protein N fail to increase with the increase in sulphur supply.

Sulphur: Between the 0.1 and 200 levels of SO_4 supply there occurred in

the leaves a 100 fold gain in sulphate, a threefold gain in soluble organic, and a twofold gain in protein sulphur. The tendency for the cotton plant to accumulate sulphate in its leaf cells (but not in stem cells) seems quite remarkable. Elsewhere (**6**) it has been shown that the leaf sap of cotton

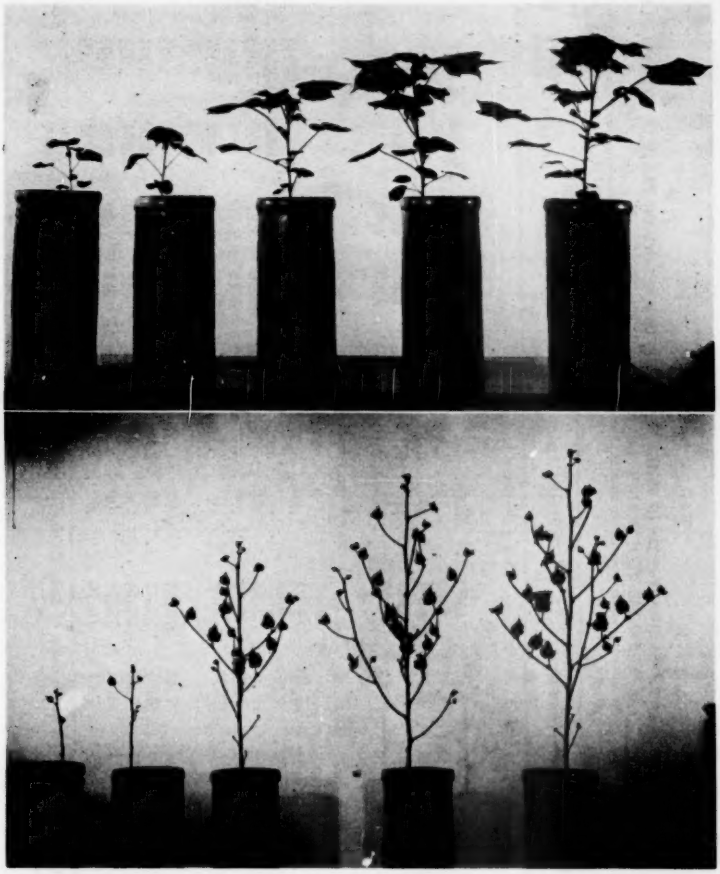


FIG. 1. Cotton plants at 35 days (above) with 0.01, 1, 10, 50 and 200 p.p.m. (C) SO_4 and (below) at 65 days defoliated.

grown on a solution containing 250 milliequivalents per liter SO_4 (3,933 p.p.m. of S) has a 0.44 normal concentration of total S expressed as sulphate.

The data of table IV are rearranged in table V to bring out certain sulphur and protein relations. The chloroplasts of the leaves of sudan grass

TABLE IV

DRY WEIGHT IN GRAMS PER PLANT AND COMPOSITION IN PERCENTAGES ON DRY WEIGHT OF PARTS OF COTTON PLANT SUPPLIED WITH VARIOUS LEVELS OF SULPHATE

SO_4 supply p.p.m.	Dry weight*	Carbohydrates				Nitrogen					Sulphur			
		Reduc. sugar	Sucrose	Starch	Hemicellulose	Nitrate	Amino	Soluble organic	Protein	Total	Sulphate	Soluble organic	Protein	Total
35 days—early bud stage														
Tops														
0.1	1.0	0.00	0.00	2.43	6.02	1.95	....	1.55	1.39	4.89	0.004	....	....	0.132
1	1.6	0.00	0.00	5.92	7.19	1.55	0.41	1.19	2.10	4.84	.005	....	....	.146
10	4.0	1.83	1.06	6.74	6.86	.92	0.17	.73	2.43	4.08	.080	....	....	.286
50	5.0	1.96	.56	6.14	7.13	.78	0.19	.73	2.56	4.07	.341	....	....	.542
200	6.8	2.11	.51	6.45	7.09	.77	0.18	.64	2.68	4.09	.837	....	....	1.057
65 days—early boll stage														
Leaves**														
0.1	1.1	0.00	0.00	7.60	2.64	1.39	0.57	2.23	0.96	4.58	0.003	0.014	0.094	.111
1	2.4	0.00	0.00	7.36	2.70	1.37	....	2.21	1.28	4.86	.003	0.014	0.101	.118
10	3.4	1.10	0.44	11.31	2.86	.06	.37	1.19	2.56	3.81	.009	0.022	0.147	.178
50	4.7	1.25	1.81	9.81	2.72	.00	.17	.51	3.25	3.76	.096	0.043	0.213	.352
200	4.7	1.32	2.11	10.34	2.38	.10	.16	.45	3.20	3.75	.362	0.047	0.198	.607
Main stalks														
0.1	0.5	0.00	0.00	7.43	13.84	0.97	1.01	3.11	.79	4.87	.000	.002	.036	.038
1	1.9	0.00	0.00	6.91	13.83	.60	1.01	2.98	.38	3.96	.000	.001	.034	.035
10	10.4	4.84	1.05	9.42	15.44	.26	.06	.39	.38	1.03	.001	.001	.040	.042
50	14.5	5.73	2.02	6.42	15.75	.08	.04	.30	.41	.79	.008	.007	.049	.064
200	13.9	5.96	1.90	7.62	15.70	.07	.02	.28	.41	.76	.011	.008	.077	.096

* Fresh weight (grams) of entire tops at 65 days of 0.1, 1, 10, 50, and 200 p.p.m. SO_4 treatments respectively: 13, 50, 237, 350, 345.
** Upper 5 per plant.

beyond the early stage of development have been found by HANSON *et al.* (11), in keeping with work on other plants, to contain upwards to 40% of the total leaf protein. It was also found that the chloroplasts contained in the order of 70% of the total protein sulphur of the leaves. The data of table V indicate that under conditions of sulphur deficiency the leaf proteins are much richer in sulphur than they are when the sulphur supply is ample; thus implying that under sulphur starvation and partial chlorosis the chloroplasts have, so to speak, a first call on the available sulphur. This idea is apparently supported by the finding that the stem proteins (storage with few chloroplasts) increased in their sulphur content from 0.73 to 3.00% as the SO_4 supply increased, whereas the leaf proteins decreased from 1.57 to 0.94% sulphur.

TABLE V

PROTEINS AND SULPHUR CONTENT OF PROTEINS IN LEAVES AND STEMS OF COTTON PLANTS AT 65 DAYS

Sulphate supply, p.p.m.	Leaves		Stems	
	Proteins (N × 6.25)	Sulphur in proteins	Proteins (N × 6.25)	Sulphur in proteins
	%	%	%	%
0.1	6.00	1.57	4.94	0.73
1	8.00	1.26	2.38	1.42
10	16.00	0.92	2.38	1.68
50	20.31	1.05	2.56	1.91
200	20.00	0.94	2.56	3.00

Discussion

As indicated in the introduction, the procedure followed in the present study of the sulphur nutrition of cotton has features in common with those used by EATON in his studies with the soybean (8), the sunflower (9), and black mustard (10). The latter studies, in turn, followed along lines similar to the earlier work of NIGHTINGALE *et al.* (14) on the sulphur metabolism of the tomato. In all cases analyses were made of stem tissues and usually the concentrations of various carbohydrates and nitrogen, as well as sulphur fractions were determined. An opportunity is thus provided for evaluating the sulphur reactions of the cotton plant in terms of the comparative physiology of the five species. Points common to the five plants provide for generalizations, and the differences provide illustrations of variations which may occur in reaction paths. The tabulations of the foregoing in table VI furnish the principal basis for the review.

Before reviewing the data of table VI, it should be noted that each of the investigators selected or developed a different base nutrient. The contrasts in the concentrations and proportions of ions, as well as differences in the frequency of solution renewal, may account for some of the variations in plant responses which might not have been experienced had all the spe-

TABLE VI

CHARACTERISTICS OF TOMATO (13), SOYBEAN (8), SUNFLOWER (9), BLACK MUSTARD (10), AND COTTON PLANTS GROWN ON SULPHATE DEFICIENT SOLUTIONS RELATIVE TO HIGH SULPHATE SOLUTIONS. THE CHEMICAL DATA ARE FOR THE PLANT STEMS EXPRESSED AS PERCENTAGES OF DRY WEIGHT

	Cotton	Tomato	Soybean	Sunflower	Black mustard
Weight reduction	95%	substantial	35%	75%	60%
Yellowing of leaves	all	lower leaves first	upper leaves first	all	upper leaves first
Leaf deterioration	lower leaves	....	lower leaves	....	....
Stem elongation	reduced 60%	normal	reduced slightly	reduced 33%	reduced 60%
Stem thickening	reduced	reduced	reduced	reduced	reduced
Sulphur in stems:					
Soluble in alc.	reduced 75%	increased	....	increased	reduced 75%
Protein	reduced 50%	reduced	....	large reduction	none present
Total organic	reduced 55%	slight reduction	unchanged	large reduction	reduced 75%
Nitrogen accumulation:					
NO_3	greatly increased	high	usually increased	slight increase	reduced
Sol. organic	greatly increased	increased 40%	great increase	large increase	increased
Protein	increased 90%	slightly higher	30% increase	20% reduction	reduced
Carbohydrate accumulation:					
Reducing sugars	reduced to 0	"high"	large reduction	reduced 75%	great reduction
Sucrose	reduced to 0	"high"	decrease	reduced 75%	great increase
Starch, dextrin.	no change	"high"	moderate increase	increased 50%	great increase
Hemicellulose	reduced slightly	....	little change	increased 50%	some increase
Minerals:					
Potassium	no change	low	....	....	....
Calcium	increased 50%	increased	....	....	....
Magnesium	increased 30%	....	....	....	....
Phosphorus	increased 40%	....	....	....	....

cies been given like substrates and like environments. By reason of these variations in growth conditions, similarities in the plant responses gain weight as being primary, whereas the departures may lose some of their significance. In all the investigations C.P. salts were used. The minus S culture solutions could not have been alike as regards the level of SO_4 supply. Nor could they have remained alike very long when subjected to SO_4 uptake by the plants, particularly in view of differences in frequency of solution renewal.

Of the five species, the greatest growth depression in minus S solutions was shown by cotton which was followed by sunflower. It seems probable that the sulphur requirement of cotton is especially high. This conclusion is indicated by the fact that even the 1.0 p.p.m. SO_4 solution (table V) gave an 85% reduction in growth which is greater than that found for sunflowers in EATON's (**9**) minus S solution.

The leaves of the cotton and sunflower were yellow from the base to the apex of the plants, without significant variation in intensity. There was yellowing in some of the leaves of tomato, soybean, and mustard but this symptom developed in the younger leaves late in the growth of these plants. CHAPMAN and BROWN (**3**) have noted the same characteristic in citrus. Yellowing of leaves, accompanied by growth depression, is in all probability the most general symptom of sulphur deficiency. In cotton leaves, the yellowish green color extends over the entire blade without gradation between the veins and outer mesophyll. It seems probable that this may also be a general characteristic in the other plants, otherwise the pattern of the yellowing would have been called to attention. Nightingale *et al.*, and Eaton directed attention to the similarity between the leaf color of minus S plants and that of plants low in nitrogen; this observation applies also to cotton. The fact that yellowing developed in the upper and younger leaves of soybean and mustard after considerable growth may indicate only that the SO_4 of the nutrients did not reach a sufficiently low level to cause yellowing until the plants were advanced in size and the SO_4 demand had become heavy. The yellowing of the lower leaves of the minus S tomato, in advance of the upper leaves, provides evidence in favor of the contention of Nightingale *et al.* that sulphur can be withdrawn from old tissues for re-use in meristems. On the other hand, if proteolysis is involved in sulphur deficiency, then in soybean and mustard it must be very slow. In the instance of these latter plants, no mention is made of a loss of chlorophyll in lower leaves at the time the yellow leaves were developing near tops of the plants. Nightingale *et al.* make a point of the observation that the yellowing of the older tomato leaves was not associated with a change in the appearance of the protoplasm.

Abscission of the old leaves of minus S cotton plants, including the cotyledons, was usually preceded by the development of necrotic margins and irregular dark colored dead areas in the mesophyll. Eaton notes for the soybean that there was a tendency for the lower leaves, especially in the

minus sulphur plants, to die and fall off. The area of four minus S soybean leaves was found to be only half that of four plus S leaves. The differences in leaf areas, were similarly marked in cotton and are indicated also in the photographs of tomato, sunflower, and mustard included in the publications cited.

Inasmuch as the longevity of a leaf must be conditioned by its physiological status, it seems doubtful that yellowing, or a shorter life, of leaves even though they are low in some mineral element, is conclusive evidence of the withdrawal of a deficient element. Expressed as percentages of green matter Nightingale *et al.* show little difference in the total sulphate free S between either the upper or lower stems or between the lower blades of plus S and minus S tomato plants, but there was a contrast between the upper blades in the two solutions.

Nightingale *et al.*, as does KRAYBILL *et al.* (**13**), note little if any effect of sulphur deficiency on the elongation of tomato stems. A slight shortening is noted on soybean, a substantial shortening for sunflower. There were marked effects of sulphur deficiency on the elongation of mustard and cotton. In all plants the stems were thin and inclined to be woody.

Of the five species, only the soybean failed to show some reduction in the percentage of total organic sulphur. Tomato and sunflower had increased, rather than reduced, the percentages of alcohol-soluble sulphur. There were reductions in protein S in cotton, tomato, and sunflower, but mustard showed no change. Mustard seems to be unique in that Eaton found it to contain no alcohol-insoluble sulphur (protein sulphur).

Possibly because of differences in the amount of woody elements, as well as inherent differences between plants, the sulphur concentration in the stems of the five plants provide a poor index to sulphur requirements. The stems of minus S cotton, dry weight basis, contained 0.04% of total organic sulphur; tomato stems (mean of upper and lower segments) 0.12%; soybean stems 0.05%; sunflower (mean of upper, middle, and lower) 0.32%; and mustard stems 0.18%. Cotton and sunflower thus had respectively the lowest and highest concentrations of organic sulphur in their stems, and yet these two plants showed the greatest growth reductions. Organic sulphur percentages of leaves of minus S plants are reported in three instances: upper main stalk cotton leaves contained 0.11%, upper tomato leaves, 0.15%, and all leaves of soybean, 0.24%. The tomato, which of these three plants had the most organic sulphur in its stems, had next to the least in its leaves. The organic sulphur of cotton leaves was three times that of the stems, the tomato approximately equal, and the soybean five times. Furthermore, it seems especially noteworthy that so little difference should have been found in the total organic S content of the stems between the plus S and minus S treatments in both tomato and soybean plants. The conclusion is indicated that between species the relative sulphur content of neither stems nor leaves provides an index to growth requirements.

Although it is reasonable to assume and believe that proteolysis and the

re-use of sulphur occurs in S deficient plants, such a relation has been and may continue to be difficult to demonstrate. Not only is this the case because of translocation and the change in the weight of tissues during periods of darkness, but also because of the recent evidence by Wood and Cruickshank (16) that the sulphur containing amino acids, cystine in particular, are broken down in the cytoplasm during protein hydrolysis; amounts of sulphate equivalent to the sulphur containing amino acids appeared.

With only occasional exceptions, sulphur deficiency resulted in higher concentrations of the soluble forms of nitrogen in all tissues and plants. But no general rule can apply to protein formation. In the stems of cotton and soybean, protein concentrations were increased, in tomato there was little change, whereas in sunflower and mustard small reductions were indicated.

Nightingale *et al.* report both reducing and sucrose sugars to have been high in minus S tomato stems. Eaton found the sucrose of mustard to show a great increase. Sugars were reduced in soybean and sunflower and in cotton little or none could be found. Starch and hemicellulose, on the other hand were usually either increased or unchanged. Three tendencies of the minus S plants stand out in the foregoing: Sugars were depressed in three of the plants, starch and hemicellulose remained high, and soluble organic nitrogen increased. The low sugar effect was especially pronounced in cotton, and the increased concentration of soluble organic nitrogen was nearly as striking (table VII). If each nitrogen atom given in this table is assumed to have been associated with four C atoms and with a hydroxyl or another group, then it is apparent that the concentration of soluble organic constituents of the minus S tissues were several times greater than in the plus S tissues.

With the question in mind of whether sugars might be present in the leaves and stems of S deficient plants at night, samples were taken on August 28 from a few plants grown outdoors on the 0.1 SO_4 solution. The following results were obtained:

	1 P.M.	8 P.M.	2 A.M.
Leaves			
Total sugars	0.47	0.36	0.05
Starch	2.69	2.84	1.33
Stems plus petioles			
Total sugars	0.01	0.01	0.00
Starch	1.19	1.85	.75

It may be noted that appreciable sugars were not found at 2 A.M. in either the leaves or stems. But sugars were found in relatively low concentrations in the leaves at both 1 P.M. and 8 P.M. It remains unknown whether the presence of leaf sugars in this test, as distinct from those preceding it in the greenhouse, is attributable to the outdoor light intensity or to some less tangible factor.

In the low sulphate cotton plants: (1) Sugars were absent, with the exception just noted, in leaves. (2) Sugars in the stems were about one fifth normal in one experiment (table II) and absent in a second (table IV). (3) Sugars in the roots were normal. (4) Starch levels were nearly normal in leaves, stems and roots. (5) Threefold, or higher, concentrations of soluble organic nitrogen were found in leaves, stems, and roots. In view of these relations it seems unlikely that much sugar could have been translocated from the leaves for the formation of the starch and hemicellulose found in the stems; the possibility is thus indicated that the primary carbon transfer was in the form of carbon-nitrogen compounds. The amino nitrogen, as well as the total soluble organic nitrogen was especially high in the low sulphate plants. The chemical data indicate that in sulphur deficient cotton plants the sugar equilibriums are shifted strongly toward the amino acids and other organic N compounds on the one hand and toward starch

TABLE VII

TOTAL SUGARS AND SOLUBLE ORGANIC NITROGEN OF COTTON EXPRESSED AS PERCENTAGES OF DRY WEIGHT—TAKEN FROM TABLE IV

SO_4 supply p.p.m.	35 days		65 days			
	Tops		Leaf blades		Stalks	
	Total sugars	Soluble organic N	Total sugars	Soluble organic N	Total sugars	Soluble organic N
0.1	.00	1.55	.00	2.23	.00	3.11
1	.00	1.19	.00	2.21	.00	2.98
10	2.89	.73	1.54	1.19	5.89	.39
50	2.52	.73	3.06	.51	7.75	.30
200	2.62	.64	3.43	.45	7.86	.28

and hemicellulose on the other, with the sugars existing only as transitory compounds in concentrations too low to give measurable reducing activity.

CHAPMAN and BROWN'S (3) results on the effect of sulphur deficiency on mineral accumulation provide background material for the present results with cotton. In orange leaves they found S deficiency to increase the percentage content of K, P, and Mg, as well as N, whereas Ca was reduced. In cotton, K showed little or no gain and Ca and Mg as well as P were increased. It would thus seem that in mineral relations, as well as in some other respects, a uniformity of reaction to S deficiency does not exist between species. The yellowing of cotton leaves evidently does not involve an iron relation; the leaves of cotton on plus S contained 0.023% iron and those on minus S 0.026%.

Summary

Cotton plants were grown on nutrient solutions low in sulphur. The leaves were chlorotic with symptoms resembling nitrogen deficiency. The stem elongation was retarded and leaf size reduced. The old leaves deteriorated.

Vegetative growth and number of bolls were increased proportionately with increasing sulphur supply, *i.e.*, there was no differential effect of sulphur on the two growth activities.

With sulphur deficiency, the concentrations of reducing sugars and sucrose in the leaves, and sometimes in the stems, were characteristically too low for measurement by the usual analytical procedures. Starch in all tissues was reduced only moderately if at all, and hemicellulose not at all.

Nitrate and soluble organic nitrogen accumulated in high concentrations; the increase in the latter was in the same order as the reductions in sugars. Protein nitrogen was greatly reduced by S deficiency in the tops of young plants and in the leaves of plants 65 days old. In the stems more protein was found in minus S than in high S plants.

Although S deficiency greatly decreased the amount of protein in the leaves, the percentage of S in this protein was higher, indicating that an unusually large part of the protein sulphur was localized in the chloroplasts.

The absence of sugars in the leaves of the low sulphate plants suggests that carbon was translocated from leaves to stems primarily in the form of organic nitrogen compounds and also that the sugars were transitory with the equilibriums shifted strongly towards amino acids on the one hand and towards starch on the other.

The discussion of the data is in a large part on the basis of the reactions of cotton to sulphur deficiency in comparison with the results of others who worked with tomato, soybean, sunflower, and mustard. A table summarizing the salient results with the five plants is presented. There were noteworthy differences in the reactions of the five plants.

In cotton, S deficiency resulted in extra accumulations of phosphorus, calcium, and magnesium. The accumulations of iron and potassium were changed little.

Little tangible symptomatic or biochemical evidence could be found of proteolysis and the re-use of sulphur. The biochemical difficulty of demonstrating sulphur re-use is indicated.

DIVISION OF COTTON AND OTHER FIBER CROPS AND DISEASES
U. S. DEPARTMENT OF AGRICULTURE
AND
DEPARTMENT OF PLANT PHYSIOLOGY AND PATHOLOGY
TEXAS AGRICULTURAL EXPERIMENT STATION
COLLEGE STATION, TEXAS

LITERATURE CITED

1. ASSOCIATION OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. Fourth Edition. 1935.
2. BEESON, K. C. The mineral composition of crops with particular reference to the soils in which they were grown. A review and compilation. U. S. Dept. Agr. Misc. Pub. **369**: 1–164. 1941.
3. CHAPMAN, H. D. and BROWN, S. M. Effects of sulphur deficiency of citrus. Hilgardia **14**: 185–201. 1941.

4. Cooper, H. P. Nutritional deficiency symptoms in cotton. Soil Sci. Soc. Amer. Proc. **4**: 322–324. 1939.
5. Cooper, H. P. and Mitchell, J. H. Chemical composition of the cotton plant grown on different soil types. Soil Sci. Soc. Amer. Proc. **2**: 374–377. 1946.
6. Eaton, F. M. Toxicity and accumulation of chloride and sulphate salts in plants. Jour. Agr. Res. **64**: 357–399. 1942.
7. Eaton, F. M. and Egrle, D. R. Carbohydrate accumulation in the cotton plant at low moisture levels. Plant Physiol. **23**: 169–187. 1948.
8. Eaton, S. V. Influence of sulphur deficiency on the metabolism of the soybean. Bot. Gaz. **97**: 66–100. 1935.
9. Eaton, S. V. Influence of sulphur deficiency on metabolism of the sunflower. Bot. Gaz. **102**: 536–556. 1941.
10. Eaton, S. V. Influence of sulphur deficiency on metabolism of black mustard. Bot. Gaz. **104**: 306–315. 1942.
11. Hanson, E. A., Barrien, H. S., and Wood, J. G. Relations between protein nitrogen, protein sulphur and chlorophyll in leaves of Sudan grass. Australian Jour. Exptl. Biol. and Med. Sci. **19**: 231–234. 1941.
12. Harris, H. C., Bledsoe, R. W., and Calhoun, P. W. Responses of cotton to S fertilization. Jour. Amer. Soc. Agron. **37**: 323–329. 1945.
13. Kraybill, H. R. and Smith, T. O. Plant metabolism studies. New Hampshire Station, Bul. 212. 1924.
14. Nightingale, G. T., Schermerhorn, L. G., and Robbins, W. R. Effects of sulphur deficiency on metabolism in tomato. Plant Physiol. **7**: 565–595. 1932.
15. Wilcox, L. V. Determination of potassium by means of an aqueous solution of trisodium cobaltinitrite in the presence of nitric acid. Ind. and Eng. Chem., Anal. Ed. **9**: 136–138. 1937.
16. Wood, J. G. and Cruickshank, D. H. The metabolism of starving leaves. V. Changes in amounts of some amino acids during starvation of grass leaves; and their bearing on the nature of the relationship between proteins and amino acids. Australian Jour. Exptl. Biol. Med. Sci. **22**: 111–123. 1944.
17. Yoe, John H. Photometric Chemical Analysis. Vol. 1. John Wiley & Sons, Inc., New York. 1928.
18. Younge, O. R. Sulphur deficiency and its effect on cotton production on coastal plain soils. Soil Sci. Soc. Amer. Proc. **6**: 215–218. 1941.

FURTHER THEORETICAL CONSIDERATIONS OF MODES OF EXPRESSION AND FACTORS POSSIBLY CONCERNED IN THE MOVEMENT OF MATERIALS THROUGH A TWO-PHASED SOLUTION SYSTEM

T. C. Broyer

(WITH TWO FIGURES)

Received July 31, 1950

Introduction

As a means of expression and schematic representation of processes and influences effective in plant physiology, investigators from time to time have sought the best modes of presentation in accord with advance of knowledge through further facts. The present work is an attempt to evaluate our present modes of expression in the field of solvent and solute movement into plants and to consider further factors possibly involved in the flux.

Throughout this discussion reference is made to the general relationship between the specific free energies and the partial molal free energies. This relationship is employed as before (**2, 3, 4, 5**) *viz.*,

$$F = \mp p - p^{\circ} = \frac{\pm k(\bar{f} - \bar{f}^{\circ})}{\bar{v}}, \qquad (1)$$

where F for either component in solution is the specific free energy in liter atmospheres per liter of the constituent component translocated; $(\bar{f} - \bar{f}^{\circ})$ is the partial molal free energy difference in solution, in ergs; k is the conversion factor from ergs to liter atmospheres; $\bar{v}$ is the partial molal volume of a constituent component in solution, in liters; and $p - p^{\circ}$ is the pressure difference necessary to make $\bar{f}$ equal $\bar{f}^{\circ}$, in atmospheres. As before, the net influx specific free energy for either the solute or solvent component between two phases, is expressed by

$$NIF = \Sigma IF - \Sigma EF. \qquad (2)$$

In words, the net influx specific free energy is equal to the difference between the sums of the energy intensities of those influences causing an influx tendency and those causing tendency toward efflux. For any particular influence,

$$NIF = (F_e - F_i) = \mp p_e - p_i = \frac{\pm k(\bar{f}_e - \bar{f}_i)}{\bar{v}}. \qquad (3)$$

The partial molal free energy difference $(\bar{f}_e - \bar{f}_i)$ for either component between solutions in e and i, is given by

$$(\bar{f}_e - \bar{f}_i) = RT \ln \frac{a_e}{a_i} \qquad (4)$$

in which $\frac{a_e}{a_i}$ is the ratio of the effective molal compositions or activities of the constituent component whose escaping tendency is being considered.

Further, diffusion pressures (in the restricted sense) of either component between solutions of a two-phased system may be expressed by

$$P = \Delta F = k_b (C_e - C_i), \qquad (5)$$

in which P is the diffusion pressure, in atmospheres; k_b is a characteristic coefficient (**5**); and $C_e - C_i$ is the concentration difference of the component whose escaping tendency is in question, expressed in grams per liter of solution.

Where movement of the solvent component (water) is considered, another relationship for the specific free energy difference is applicable, where the solute concentrations in solution are sufficiently dilute, namely

$$(F_{s_e} - F_{s_i})_1 = -RT\,[(k_v \times c_2)_e - (k_v \times c_2)_i], \qquad (6)$$

in which subscripts 1 and 2 refer to solvent and solute respectively; k_v is the van't Hoff coefficient; and c_2 is the molar concentration of the solute species in solution.

Discussion of modes of expression

In earlier articles, various terminologies have been used as means of expressing the factors concerned in solute and water movement (**2, 3, 4, 5, 6, 10, 14, 16, 20, 24**). References **2, 3, 4,** and **5** should be consulted in particular. In general, investigators have had the same viewpoint and have merely expressed themselves in different terms. Each of these modes of expression has been useful as defined. Certain limitations appear in each case, and it is the purpose here to discuss the possible advantages and disadvantages of the proposed modes of expression.

There are certain advantages and limitations to the use of the terms specific (volumed) free energies (energy intensities) and net influx specific (volumed) free energy (net influx energy intensity) in particular. They may be favored because they express the tendencies for movement of a constituent component in solution in terms of the free energy concept. They have essentially the advantages possessed by the use of partial molal free energies. Therefore, these usages are sound energetically. Further, these tendencies for movement are expressed in dimensions quantitatively equivalent to ones which are practically measurable, namely pressures.

Some investigators have suggested possible confusion between two uses (massed and volumed) of specific free energy (**23**). The use of the alternate (parenthetic) phrase for the latter, is suggested by these authors, which if wise, involves merely an inversion of the phrases, specific (volumed) free

energy and action capacity in the definition stated earlier (**2, 3, 4,** including supplementary statements in **4,** pp. 163–164). However, the word energy should be substituted for action, and intensity (**18,** p. 13) for capacity, for dimensional accuracy. In all of these studies, the energy intensities are flux intensities. For the comparative use of free energy in contrast with entropy, or potential, see **13,** pp. 83–84; compare **12** and **18** also.

Escaping tendencies or tendencies for movement of matter are fundamentally expressed as molal (or partial molal) free energy differences (the actual free energy related to that in a standard or reference state), an intensive quantity derived from the extensive (**18,** p. 13) property of the molecular species in question by limiting the energy difference to that statistical mean value associated with the number of molecules in one mole of a constituent component (a constant = 6.06×10^{23}) of a system under the existing conditions. These theoretically measurable energy intensities are often expressed in extensive dimensions of energy, *viz.*, mL^2t^{-2} (for example, in erg units) with the limitation that the free energy difference is restricted to that of a mole of substance, thus strictly intensive. Other intensive useful work term usages may be employed in place of the molal or partial molal free energy difference, namely a free energy limited to a unit mass or volume, yielding energy intensity values designated as specific (massed or volumed) free energies. These are expressed in dimensions of either energy per unit mass mL^2t^{-2}, *e.g.*, ergs per gram; or energy per unit volume $mL^{-1}t^{-2}$, *e.g.*, liter atmospheres per liter or atmosphere units. Use of the latter dimension is preferred by some, since it is quantitatively equivalent to (though qualitatively not identical with) that of a pressure which usually can be measured by physical means at the disposal of the investigator (**1,** pp. 156–157; **18,** pp. 204–205, 235–236, 242–244). This volumed free energy is thus a practical expression while the molal (or partial molal) and massed free energies as such are of limited practical value.

The ambiguity between a pressure concept and flux intensity, which concept is probably untenable, may be resolved as follows: The volumed specific free energies are dimensionally equivalent to pressures, m/Lt^2, and are often so computed, *e.g.*, as proportional to $RT/\bar{v}$ where the proportionality coefficient may be the ratio of the relative vapor pressures or solution compositions, or as derived from lowerings of the freezing point or raisings of the boiling point; or are so measured, *e.g.*, as the pressure difference necessary to make the partial molal free energy of a constituent component in a two-phased solution system in the given state $\bar{f}$, equal to its partial molal free energy in a reference state $\bar{f}^o$. Hydrostatic or turgor pressure is one, and the only, physical manifestation equivalent to such an energy intensity in aqueous solution systems. Typical units for flux intensity are liter atmospheres per liter of constituent migration, or resultant atmosphere units. However, the volumed specific free energies are not pressures. (Compare definitions of osmotic pressure and their relation to energy intensities.) In other words, flux intensities are not forces per unit area, but rather are

forces concerned with the ordered movement of a unit volume of a constituent component of solution through a unit of distance. Volumed specific free energies are dimensionally analyzed as follows (**4**, p. 127):

F [Flux (Energy) Intensity]

$$= \frac{\bar{f} - \bar{f}^{\circ}}{\bar{v}^{\circ}} = \frac{\text{(energy per mole)}}{\text{(volume per mole)}} = \frac{\text{(force} \times \text{distance)}}{\text{(volume)}} = \text{but} \neq \frac{\text{(force)}}{\text{(area)}}$$

$$\text{Atmospheres} = \frac{\text{Liter Atmospheres per mole}}{\text{Liters per mole}} = \frac{1.013 \times 10^{6} \text{ Dyne Centimeters}}{\text{Liter}}$$

$$\frac{m}{Lt^2} = \frac{mL^2}{t^2 n} \times \frac{1}{L^3/n} = \frac{mL}{t^2} \times \frac{L}{1} \times \frac{1}{L^3} = \text{but} \neq \frac{mL}{t^2} \frac{1}{L^3}$$

The conversion of the free energy relations between dimensions of energy per unit volume and energy per mole is simply accomplished, by application of a coefficient, *viz.*, the reciprocal of the partial molal volume of a constituent component in solution, and possible change of algebraic sign. In order to obviate the awkward mathematical property of the difference in molal (or partial molal) free energy approaching minus infinities under certain circumstances, the algebraic sign of the specific free energy may be changed, where it is desirable for certain means of discussion.

The scheme is useful, by direct analogy, for discussion of the tendencies for movement of either solvent (osmotic specific free energies) or solute (solute specific free energies) through a two-phased solution system. The grouping of the tendencies for movement into categories of influx, efflux, and net influx specific free energies as in equation 2, is useful in graphically representing the changes in escaping tendency of water with changes of internal volume of an osmometer (a two-phased solution system with a semi-permeable membrane interposed).

Two limitations exist: First, the partial molal volume of a constituent component is not constant, being approximately so only where sufficiently dilute solutions are involved. However, this is objectionable only for purposes of conversion from dimensions of energy per unit volume to energy per mole. Moreover, the variation is not appreciable within the solute composition limits of solutions generally encountered in biological systems. Second, in estimating the rates of net flux, the flux coefficient k_{ϕ} (**5**) is variable with change in composition of the solutions in the two phases. Evaluation of this coefficient, for any particular case, must be derived by computation from direct measurements. Compare discussion of this flux coefficient with that of the related diffusion coefficient (**5**).

As with specific free energies, there may appear to be certain advantages or disadvantages to the use of the terms diffusion pressure (**5, 26**) or diffusion pressure deficit (**20**) in the sense of FICK (**12**). Flux of materials in all natural systems, involves diffusion in part, this tendency being a func-

tion of the relative concentrations of the constituent whose migration is in question. The term diffusion has a restricted meaning in older usage (**4**, pp. 163–164) related to a difference in concentration of a constituent component in solution. In reality, other factors are involved also, *e.g.*, temperature, pressure and galvanic differences; and physico-chemical movements of materials may often be in a direction contrary to such concentration differences alone. Substitution of the term flux for diffusion could obviate this possible disadvantage.

Where limited to diffusion phenomena in the restricted sense of Fick, the net flux intensity for either water or solute is a function of the relative compositions of the solutions in the two phases concerned. Where specific free energies are considered, the term composition here implies the effective molal composition (molal activity), and the term relative implies the logarithm of the ratio of these compositions (**5**, equations 4 and 29). Where diffusion pressures are considered, the term composition implies the concentration expressed in grams per liter of solution, and the term relative, implies the difference of these concentrations (**5**, equations 7 and 30). The diffusion pressure mode of expression is easily visualized through possible analogy to movements of gases; for water in dilute solutions, it bears a formal relationship in computation, to the gas law (equation 6). The molecular kinetic viewpoint is discussed later.

With sufficiently dilute solutions, where water movements are especially of interest, the energy intensity of water translation is indirectly equated in part to concentration differences of the solute in solution, *viz.*,

$$(Fs_e - Fs_i)_1 = -RT\,[(k_v \times c_1)_e - (k_v \times c_2)_i], \qquad (6)$$

(**5**, equation 31). The usual measurements of ΔFs_1 are expressed in dimensions equivalent to pressure. However, this resultant escaping tendency should be recognized to be manifested strictly as an energy intensity, *i.e.*, liter atmospheres per liter of water flux where expressed in atmosphere units. Provided we could satisfactorily evaluate each of the factors concerned, equations 1 and 4, or 5, should be used in place of equation 6, thus removing any ambiguity in mechanistic interpretation.

Where a difference in escaping tendency is caused only by a difference in concentration of a constituent component in solution, actual pressure (**26**, p. 193) is relatively very small;[1] that is, a simple manometer inserted into a single solution, will not record a pressure equal in magnitude to the osmotic pressure as frequently defined. Substitution of the term intensity for pressure could obviate this possible disadvantage.

Diffusion pressure deficit is frequently recognized to be the deficit of diffusion pressure of a constituent component in solution in a given state from that in a reference or a standard state, for any single modifying influence or the resultant of any number of modifying influences in a single

[1] See Guggenheim, E. A., Modern Thermodynamics (pp. 85–88, 1933) for one viewpoint. Dr. L. Machlis has drawn this work to the attention of the author.

phase; or the resultant between two phases within each of which there may be any number of modifying influences. Extension of this phrase to read: a net diffusion pressure deficit or diffusion pressure deficit difference, obviates this possible disadvantage. For water, influx is measured by positive dimensions of DPD; for a constituent solute species, comparable dimensions would have to be expressed by negative values. In other words, for a constituent solute, DPD values are of inverse sign from those applicable to the solvent.

Osmotic pressure, equal to but not identical with, the net diffusion pressure of solvent, has been defined as $p - p^o$ (**3**, p. 5; **2**, pp. 67–68) between a solution phase of an osmotic system with relation to water in the other. MEYER (**20**, pp. 151–152) has defined the term osmotic pressure, in a derived manner, as a property of a solution. Such a usage has much to be said in its favor, especially where the $(p - p_o)$ difference may be expressed as equal to the net diffusion pressure of water. The former, physico-chemical definition however is preferable (**26**).

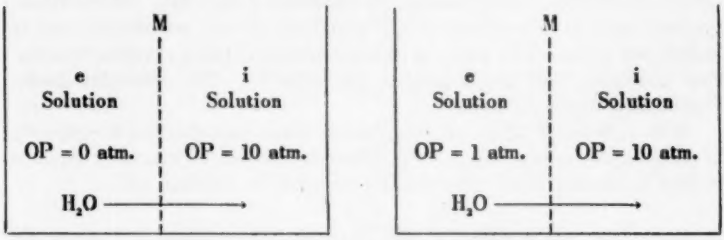


FIG. 1. Movement of water between two solution phases varying only in salt concentration.

EYSTER (**14**) has suggested use of the terms osmotic pressure and osmotic pressure deficit for certain purposes. Where based on the usual definition of OP, the terms are useful for discussion of the tendency for movement of the solvent component only. The term OP deficit, though satisfactory in itself, affords but an incomplete analysis of the net tendency for water to move through natural systems. Between two solution phases varying only in salt concentration, water moves then from the lesser, positive OP value, to the greater, positive OP value (fig. 1).

EDLEFSEN *et al.* (**13**) have suggested the use of the term specific free energy (energy per unit mass). It has essentially the advantages possessed by the thermodynamic partial molal free energy. No advantage accrues however from division throughout by the molecular weight of a constituent component in solution, except in computation of rates of energy expenditure. Here, use of the ratio $\frac{\bar{v}}{m}$ (**5**, equation 26) is unnecessary. The dimensions of this (massed) specific free energy are not directly commensurable with those of pressure, as noted by MEYER (**20**, p. 148).

The relationships between the massed (**13**) and volumed (**2**) specific free energies are given by

$$\Delta F_m \times \frac{m}{\bar{v}} \times 9.87 \times 10^{-10} = \Delta F_v \qquad (7a)$$

and

$$\Delta F_v \times \frac{\bar{v}}{m} \times 1.013 \times 10^9 = \Delta F_m, \qquad (7b)$$

in which ΔF_m is expressed in ergs per gram; ΔF_v is expressed in liter atmospheres per liter, or atmospheres; m is the molecular weight expressed in grams, and $\bar{v}$ is the partial molal volume expressed in liters, of a constituent component of solution; 9.87×10^{-10} is the conversion factor from ergs to liter atmospheres, and 1.013×10^9 is the reciprocal factor for conversion from liter atmospheres to ergs.

Certain advantages or disadvantages may attend the use of schemes and/or terminologies other than those discussed in earlier sections. For previous criticisms of various osmotic factors, compare URSPRUNG-BECK (**24**) and MEYER (**20**). One circumstance which might be considered in favor of certain older usages, *e.g.*, suction force or pressure or tension, water-absorbing capacity; or osmotic pressure, osmotic value, *et cetera*, is historic precedence. Except where possible confusion may enter in, continued use of early terminology has much to be said in its favor. Common expression makes for ready comparison of the work of various investigators. However, the use of the term osmotic value, for example, is less expressive than others proposed; compare osmotic solute specific free energy (osmotic solute energy intensity) (**3**). This term is used better, in a general sense; all osmotic quantities are osmotic values.

The recent schematic representation of osmotic factors with volume of the inner phase by BURSTRÖM (**9**, compare **6**) allows less ready summation of the scalars of influences causing water to tend to move in similar directions (inward or outward). His redefinition of turgor pressure, as a factor unrelated in magnitude (except at water equilibrium) with wall pressure, is quite fascinating. Burström suggests $T = O - E$ and $O = S + W$ as two independent definitions, where T is the turgor pressure, O is the osmotic value of the cell sap, E is the osmotic value (or suction) of the external medium, W is the wall pressure, and S is the suction of the entire cell. The value $O - E$ is the net flux intensity (net osmotic flux specific free energy) as modified only by the presence of solute in either or both of the solutions of a two-phased system. The value $O - E$ is an energy intensity difference, the effect of which, through net movement of water from the external to the internal phase, may lead to the realization of a progressively increasing internal hydrostatic solution pressure (turgor pressure, in the usual sense) and concomitant energy intensity until a steady state of dynamic equilibrium for water movement is attained, where, other osmotic factors being ineffective, $O - E = T$. A positive difference between the sums of the energy

intensities of those influences causing an influx tendency and those causing tendency toward efflux (equation 2) is the ultimate cause of any realized expansion, however, mediated through net flux of water into the ideal osmometer; the immediate cause is the positive pressure difference between the internal hydrostatic pressure (turgor pressure in the usual sense) and the external pressure. During disequilibrium this pressure difference may be assumed to differ only infinitesimally at each moment (**18**, pp. 53–54). The extent of enlargement concerns the process path and is thus determined in part by the elasticity and plasticity of the limiting boundary (**8**). The internal hydrostatic pressure is the force normal to unit area of the boundary of the internal phase. It is equal to the wall pressure where the latter is defined as the reaction to the turgor pressure (usual sense) as would be required by Newton's third law of motion. The external pressure is equal to the sum of a pressure equivalent to a force per unit area resulting from tangential stress within the boundary (boundary equivalent pressure or intrinsic external pressure) and any other possible pressure thereon of external origin (extrinsic external pressure). The stress within the boundary is a function of the elastic and plastic properties of the boundary and its strain already present. The boundary equivalent pressure is difficult to evaluate. However, since the internal hydrostatic pressure at any instant differs only infinitesimally from the external pressure, the former is generally used as a measure of the latter. The external pressure, especially in the absence of components of strictly external origin, may be conceived (though possibly less appropriately) to be a wall pressure (**6**), but dissimilar, here, to the restricted usage as defined above. The restricted usage is possibly preferable in order to avoid ambiguity. Converse causes and effects are applicable for volume contraction.

With natural systems, the various thermodynamic functions (E, F, H, etc., **18**) are all concerned. They may be evaluated as energy intensities and algebraically treated in single equational form (equation 2) without regard to their nature, for convenience. Their use depends only on the initial and final states of the system, and thus they may or may not have work significance. Work expressions generally concern the initial and final states of the system and the process path.

In general, moisture relations are of importance to the plant-soil investigator to the end that interest lies in the availability of water to the plant for its growth. Such studies involve the investigation of a two-phased osmometer system as discussed above. The soils specialist often restricts study to that on a single phase (not state), that in the soil (**22, 23, 25**). For such special situations, the scheme employed for the osmotic system is not useful and even the terminology (especially the positive or negative sign of the quantities) may require modification. To avoid confusion, it would be of course better to consider tendencies for water movement from the standpoint of the two-phased system, plant-soil, in all cases.

Use of the term specific (volumed) free energies (energy intensities)

where a single phase is considered relative to water as above, expresses the escaping tendencies of the constituent component water in physically measurable, energetically sound, dimensions. These can be expressed individually or as the resultant energy intensity. Specific free energy (energy intensity) deficits of soil water could be employed best, as positive quantities of synonymous flux intensity. These are a consequence of either an imbibitional (surface effect) or solute factor or both. Since a single phase relative to water is considered, these quantities have negative values; the energy intensities are antiphasically directed through a two-phased system. Although negative, they should not be visualized as tensions, because the resultant factor is an energy intensity and not strictly a force per unit area. The use of specific free energy (energy intensity) deficits (cf. **20**) might obviate this disadvantage.

The terms, soil moisture tension and/or total soil moisture stress have been used. They bear historic precedence and soil moisture stress in particular appears to give an expressive character to the factors involved. The term stress suggests the application of factors (imbibition and solute) whose influences on the energy intensity of water are positively applied; yet as water flux intensities, they are negatively directed. The term tension suggests an actual negative force per unit area while the quantity is an energy intensity.

A molecular kinetic pressure interpretation has been applied to the process of osmosis for pedagogical purposes. Such an interpretation is based on the concept that a kinetic pressure is effective in movement of materials, where, from the kinetic theory of gases

$$PV = \tfrac{1}{3} N\bar{e} = RT,$$

in which P is the pressure and V is the volume of the gas; N is the number of molecules; $\bar{e}$ is the average energy of the molecules (**12**, pp. 82–100); and other notations are as before. This concept may give a mental picture of what would seem to be occurring in the solutions, especially at the external and internal membrane surfaces. The migration phenomena for water are thus possibly more readily understood by students of osmosis. However, no better explanation is afforded by this interpretation, based on the assumption of kinetic pressures, than that by the fundamental consideration of free energy differences across the system, provided the student comprehends the free energy concept. Moreover, the molecular kinetic interpretation which would assume all escaping tendencies to be related to real pressures in effect, is purely a theory and has not, and possibly cannot be, experimentally demonstrated for solutions. A simpler, possibly more plausible, hypothetical interpretation may be based on the assumption that solute increase, or pressure or temperature decrease, within a phase, by some means increases the inter-molecular attractions within a phase, such that the escaping tendency of water therefrom is lowered. This viewpoint, though hypothetical, would appear to be in accord with the free energy concept.

However, even this interpretation may not apply to certain idealized conditions, *e.g.*, where intermolecular attractions between solvent molecules equal those between solvent and solute.

All energy intensities for movement are intensive free energy terms, not pressures, even though they may be so measured physically, or expressed on the volumed basis in such dimensions. Compare the dimensions of molal free energy and/or those on the massed basis, which are comparable.

Assuming for purposes of discussion that the molecular kinetic interpretation is valid, there may be certain advantages or disadvantages to a solute pressure viewpoint in discussions of osmosis. An analogy can be visualized between pressures and movement of molecules in solution, based on the van't Hoff equation $pv = nRT$; where p is the pressure within a phase, v is the volume of the phase, and n is the number of moles of the constituent in the volume v. Such an analogy, however, may be unwarranted on current concepts in physical chemistry. The assumption that molecules act similarly in gases and in solutions is debatable. There is probably but a formal resemblance between van't Hoff's osmotic pressure equation and the equation of state of a perfect gas (**12**, p. 312; **26**, p. 190; **3**, equations 1 and 3; **5**, compare **1**, pp. 156–157).

It has been considered by some, since a semi-permeable membrane is interposed between two solution phases, that the membrane is completely permeable to the solvent while completely impermeable to the solute. Thus only the solute could exert a kinetic pressure on the limiting surface. Such an idealized situation is never realized, especially in nature. Furthermore, the presence of a membrane, in fact, implies restriction to flux, through or across the boundary, for both solute and water (see **5**, on the nature of the permeability coefficient k_p). Thus is would seem that both components of solution could, if the theory holds, exert such pressure.

Similarly, there may be certain pedagogical advantages or disadvantages to a solvent pressure viewpoint in discussions of osmosis. In accord with specific free energy differences in osmosis, it may seem better, if a choice must be made for the purpose of discussion, to recognize the kinetic pressure to be related to the water component rather than to the solute in solution. By analogy among three similar influences involving possible osmosis, *viz.*, increased solute concentration in solution, decreased pressure (or increased tension), and decreased temperature, decreasing the specific free energy (energy intensity) of water, it may seem better to recognize the kinetic pressure to be related to a difference of the solvent component (greater in e than i, in each case) rather than to the solute in solution which is only necessarily present in the first case. This analogy is exemplified diagrammatically in figure 2. It may be observed, that in each case an appropriate pressure applied to phase i, at $t = 0$, will maintain the status quo of the osmotic system, *i.e.*, the initial steady state for water. Only in case (a) is the applied pressure, necessary to maintain the initial dynamic equilibrium, effective in likewise increasing in equal amount, the specific

free energy of the solute component, which could be related to a solute pressure (solute being absent in i in all other cases). From analogy with the other cases cited, however, it might seem more reasonable to visualize this to be related to a difference in pressure of the solvent.

The influences of added solute, or pressure or temperature differences on the specific free energy of solvent water and a typical solute species are discussed further in another part of this article. From these computations, it might seem, with due consideration of limitations cited above, however, that the difference in energy intensity between the two phases of a typical osmometer for water is in accord with consideration of a solvent pressure rather than a solute pressure. If a hypothetical reference cell with an internal medium containing 0.1 molar KCl is placed into an isosmotic external

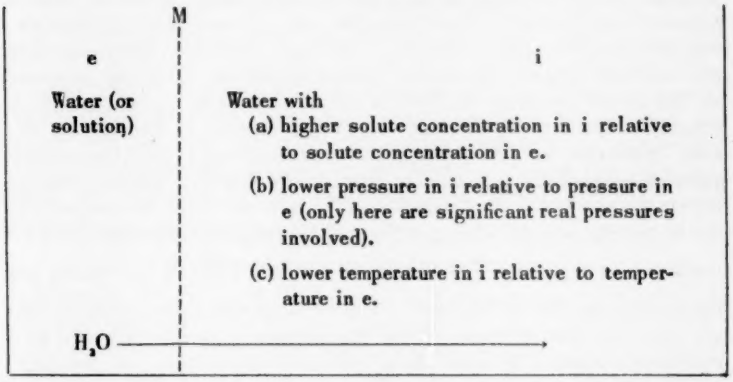

M
e
i
Water (or solution)
Water with
(a) higher solute concentration in i relative to solute concentration in e.
(b) lower pressure in i relative to pressure in e (only here are significant real pressures involved).
(c) lower temperature in i relative to temperature in e.
H_2O

FIG. 2. Movement of water as influenced by solute concentration, pressure and temperature.

medium of KCl, no net flux of water would occur across the interposed membrane; hydrostatic pressure (turgor pressure) being zero internally. If the cell is now transferred to a KCl solution of 0.001 molarity, water will tend to enter by osmosis. At $t = 0$, an imposed pressure of 4.5 atmospheres on the cell will maintain the steady state of the system, equal to a hydrostatic pressure which would tend to be established (with due correction for dilution) if no pressure were imposed, at $t = \infty$. It should be observed, that, at $t = 0$, no hydrostatic energy intensity difference exists in i, but rather, a large solute energy intensity difference (in nature, attained and possibly maintained in a living system through expenditure of metabolic energy). This latter energy intensity difference is relatively ineffective in expansion of the cell, even though very large in magnitude. Turgor is only realized on entry of water toward osmotic equilibrium with time, or imposition of a pressure to maintain the *status quo*. This real pressure is small (4.5

atmospheres), equal to the hydrostatic pressure on the membrane at the steady state, when $t = \infty$. Simultaneously, the specific free energy of internal solute is correspondingly increased (except as dilution may occur), but small in magnitude relative to that already existent as a solute energy intensity difference.

The solvent pressure hypothesis seems to be more logical, especially where solute is not involved, as in examples presented above. However, where solutions are concerned, this viewpoint may appear to fail to account for the concurrent effects of the solute. Where only water is present in closed phases, with a turgor factor included, the hydrostatic pressure at $t = 0$ is equated to the inwardly directed constriction pressure of the limiting surface. Similarly, in open phases with a hydrostatic head, the hydrostatic pressure at $t = 0$ corresponds to a similar directionally effective pressure equated to the product of the height above the reference level, the acceleration due to gravity, and the density of the water medium. Where solutions are concerned in closed phases, the pressure in solution (generally expressed as hydrostatic pressure—but not so restricted in fact) again at $t = 0$, is equated to the inwardly directed constriction pressure of the limiting surface. Similarly, in open phases with a static column ($t = 0$), the solution pressure corresponds to a similar directionally effective pressure equated here to the product of the height above the reference level, the acceleration due to gravity, and the density of the solution medium. The density of the medium here is equal to $\frac{m}{v}$, where m is the mass and v is the volume per unit of solution (**18**, pp. 242–244). The solute as well as the solvent is concerned in the density value. While the pressure is usually expressed as a hydrostatic pressure, it is obviously related to the solution. At the reference membrane surface the solution pressure causes both solute and solvent to tend to move independently across the surface. The translational energy intensity thus arising, is equally effective for both components of solution. On the molecular kinetic viewpoint, the kinetic energies of both solute and solvent would be increased. The effects are independent; as solute or solvent pressures they are not additive, being an intensive property of the solution, the increased tendency for flux of each component existing simultaneously. Similar escaping tendencies could arise through temperature or mechanical pressure differences.

In summary of the molecular kinetic viewpoint in discussions of osmosis, it should be recognized, therefore, that the kinetic pressures of the solvent and the solute, though acting simultaneously and generally not equal in magnitude, are not additive but independent in transport effect. This statement relates to the energetics of flux alone, although physico-chemical influences between the two components and modification due to temperature or pressure (including hydrostatic or solution) lead to altered energy intensities of each in solution. Thus, if the kinetic viewpoint must be adopted for

discussion purposes, the net tendency toward movement through a two-phased solution system for water, it would seem, should be related to the resultant kinetic pressure of this component; for solute, to its resultant kinetic pressure. Finally, it may seem wiser, as stated earlier, to avoid any reference to either solute or solvent pressure as effective *per se* in causing tendency toward solute flux or osmotic movement of water. Rather, it should be urged that the viewpoint of independent free energies of constituent components of the system be considered. Based on free energy differences, flux of either component is rationally and completely expressed, and adequately visualized in terms of specific free energies or energy intensities of translation.

Diagrammatic representation of possible modes of migration of solutes and water in solution

It is often helpful, as an aid in discussion, to have some diagrammatic scheme for reference. All detailed theories of flux cannot be expressed fully in a single diagram. Several mechanisms, especially concerned with solute migration, have been schematically represented by proponents of each. An attempt has been made (**7**) to present a diagram which can be used generally, for visualizing and discussing the movements of both solutes and water into cellular systems. This will be presented elsewhere.

Discussion of various factors concerned in the specific free energy relations of water and solutes in solution

In earlier articles (**2, 3, 4**) analyses and discussions of a number of factors generally concerned with the movement of materials through a two-phased solution system were presented. Those that were treated have been generally recognized as important under most conditions. Several influences were not discussed specifically, but questions often arise as to their significance in the over-all process of solvent and solute flux. These are evaluated here, where possible, in order to recognize whether they should be generally considered or whether they are of insignificant importance in systems usually dealt with. A comparative evaluation for dilute solutions is made of the effects of: differences of 1) solute concentration, 2) pressure, 3) temperature, and 4) electromotive force between two phases of an osmometer on the differences in specific free energy of a constituent component (water or solute) of the system; 5) the escaping tendency from curved surfaces; 6) influences of solutes, temperature, and pressure on surface tensions as concerned with the specific free energy of water in solutions.

Solute effects

For a KCl concentration difference between two solution phases, of for example $i = 0.1$ molar and $e = 0.001$ molar, the specific free energy differences of the water and of the solute respectively, are:

Water:

Given $\bar{v} = 0.018$ liters

$T = 25°C = 298°$ Absolute

$R = 0.0821$ liter atmospheres per degree

$k_v(0.1\,m) = 1.86$ (osmotic equivalent coefficient)

$k_v(0.001\,m) = 1.98$ (osmotic equivalent coefficient)

$F_i = -(k_v \times c_2 \times RT)$

$Fs_{1i} = -(1.86 \times 0.1 \times 0.0821 \times 298) = -(1.86 \times 0.1 \times 24.45) = -4.55$ atm.

$Fs_{1e} = -(1.98 \times 0.001 \times 0.0821 \times 298) = -(1.98 \times 0.001 \times 24.45) = -0.0485$ atm.

Therefore, $(Fs_{1e} - Fs_{1i})_{TPG} = -0.0485 + 4.55 = 4.50$ atm.

Or, $(Fs_{1e} - Fs_{1i})_{TPG} = -RT[(k_v \times c_2)_e - (k_v \times c_2)_i]$

$= -24.45\,[(1.98 \times 0.001) - (1.86 \times 0.1)]$

$= 4.50$ atm., inwardly directed (compare with direction for solute below). Subscripts T, P, G denote constancy of temperature, pressure, and galvanic factors, respectively, across the interposed interfacial surface, at $t = 0$.

Solute (KCl):

$$(Fs_{2i} - Fs_{2e})_{TPG} = \frac{(2.303)\,RT}{\bar{v}} \times \log \frac{a_{2i}}{a_{2e}}$$

$$= \frac{2.303 \times 0.0821 \times 298}{0.02} \times \log \frac{0.1 \times 0.794}{0.001 \times 0.922}$$

$$= 2.815 \times 10^3 \log 86.1$$

$$= 2.815 \times 10^3 \times 1.935$$

$= 5.34 \times 10^3$ atm., outwardly directed.

Metabolic specific free energy (inwardly directed) of accumulation is generally effective in nature, in counteracting this energy intensity. In the above analysis a_i and a_e are the activities of the solute in the two phases. The activities or effective (corrected) compositions are computed from the relations $a = \gamma c_2$ in which a is the activity, γ is the activity coefficient, and c_2 is the molal composition of the solution (**18**). Here the molal compositions are assumed to approximate the molar concentrations, *i.e.*, the densities of the solutions in i and e are assumed to approach unity. Compare LEWIS and RANDALL (**18**, chap. 28) on reaction equilibria, and OLSON and SIMONSON (**21**) on reaction rates. The latter have raised the question as to whether reaction rates in mixed electrolyte media are related to the molal activities of the reactant ions as dependent on the ionic strength. In their studies, the rates were functions rather, of the concentrations of the reactant ions. It may seem debatable as well, at this time therefore, whether the rates of flux in studies with inanimate colloidal and living systems are expressed better as a function of the concentrations or activities as usually employed (compare **11** and **19**). Although the rates of movement may be

so delimited, *i.e.*, as a function of concentration, the specific free energy as a measure of the escaping tendency, is retained here, as usually expressed, *viz.*, proportional to the logarithm of the ratio of the activities. Such usage maintains composition *per se* of solutions, independent of temperature and/or pressure.

Pressure effects

The specific free energy differences of both solvent and solute components are modified directly by pressure differences, equal in effect to the existing differences, *i.e.*,

$$(F_{p_{1i}} - F_{p_{1e}})_{MTC} = (p_i - p_e) \tag{8}$$

$$(F_{p_{2i}} - F_{p_{2e}})_{MTC} = (p_i - p_e) \tag{9}$$

These effects are concerned only where the pressure difference is between phases of a two-phased system. Changes in pressure in systems as a whole, while modifying the escaping tendency of both components in both phases, do not modify the resultant energy intensity of either component between phases. It should be noted, that while escaping tendency of a pure substance must increase with pressure, this is not true for a substance in solution, where v may be negative (**18**, p. 204).

Temperature effects

Two separate effects of temperature are possible: first, change of temperature of the system as a whole on the resultant energy intensity for translation of either water or solute component, and second, a difference of temperature between the two phases of the system. For two systems of similar concentration difference between phases, at two different temperatures (temperature constant between phases) the energy intensities for movement of water and solute are given by:

Water:

$$\Delta F_{s_1} = RT(k_v c_i - k_v c_e) \tag{6}$$

At 25° C

$$\Delta F_{s_1} = 0.0821 \times 298\ (1.86 \times 0.1 - 1.98 \times 0.001) = 4.500 \text{ atm.}$$

and At 26° C

$$\Delta F_{s_1} = 0.0821 \times 299\ (1.86 \times 0.1 - 1.98 \times 0.001) = 4.515 \text{ atm.}$$

These energy intensities are both inwardly directed. The effect of a change of one degree rise in temperature of the system, is to increase the net influx intensity by 0.015 atmospheres. Larger changes of temperature will proportionally increase the resultant migration tendency, *i.e.*, here, by 1.5×10^{-2} atmospheres per degree change in temperature of the system. It may be noted that the absolute and percentage changes in energy intensity difference are rather small for the temperature range effective in nature.

Solute:

$$\Delta Fs_2 = \frac{RT}{\bar{v}} \ln \frac{a_i}{a_e} \qquad (10)$$

At 25° C

$$\Delta Fs_2 = \frac{2.3 \times 0.0821 \times 298}{0.02} \times \log \frac{0.1 \times 0.794}{0.001 \times 0.922}$$

$$= 2.815 \times 10^3 \times 1.935 = 5.44 \times 10^3 \text{ atm.}$$

and at 26° C

$$\Delta Fs_2 = \frac{2.3 \times 0.0821 \times 299}{0.02} \times \log \frac{0.1 \times 0.794}{0.001 \times 0.922}$$

$$= 2.82 \times 10^3 \times 1.935 = 5.45 \times 10^3 \text{ atm.}$$

The energy intensities are both outwardly directed. The effect of a change of one degree rise in temperature of the system, is to increase the net efflux intensity by 10 atmospheres. Larger changes of temperature will proportionately increase the resultant flux tendency, *i.e.*, here, by 10 atmospheres per degree change in temperature of the system. It may be noted, that while the absolute change in energy intensity difference is large compared with that for water, the percentage increase is small and of the same order as that for the solvent.

The immediately preceding discussion has been concerned with effects of temperature change on a two-phased system as a whole. Since the free energy function is designed primarily for the study of isothermal processes, the problem of effects of differences of temperature between phases of the system cannot be solved by these functions. By other means, an approximation of the importance of these effects can be obtained. Since in natural systems, the difference in temperature between phases is probably very small, these effects may be considered of little importance for either water or solute. That they exist, has been shown by the Soret effect (**12**, p. 244; **18**, p. 184, pp. 147–149).

Electromotive force (galvanic) effects

The influences of galvanic differences are largely open to controversy. Little is known concerning the evaluation of junction potentials under practical conditions. Although the processes of electro-osmosis and cataphoresis are recognized as possibly involved in the net flux of materials into cells, it is likewise unknown whether the potentials often measured are the cause in part of flux of substances or are the result of such movement. Further, no direct means of computation is known for evaluation of possible effects of electromotive force differences between two solution phases. One indirect procedure is available, but it must be admitted that the significance of such

a computation for electrolyte migration is completely speculative. The computation may be made in the following manner:

Solute (KCl):

The partial molal free energy of a constituent electrolyte component is given by the relation

$$(\bar{f}_{2i} - \bar{f}_{2e}) = RT \ln \frac{a_{2i}}{a_{2e}} = -NF\,(E)_{i-e} \qquad (11)$$

(18, p. 333), where N is the number of equivalents per mole of electrolyte. F is the Faraday constant (here, 953.0 liter atmospheres per volt equivalent), and $(E)_{i-e}$ is the existing voltage difference. For KCl, the partial molal free energy difference per millivolt difference then becomes:

$$(\bar{f}_{2i} - \bar{f}_{2e}) = -NF\,(E)_{i-e} = -1 \times 953(-0.001) = +0.953 \text{ liter atmospheres.}$$

The corresponding specific free energy difference of the solute is

$$(\Delta Fg_2)_{MP} = \frac{(\bar{f}_{2i} - \bar{f}_{2e})}{\bar{v}} = \frac{0.953}{0.02} = 47.6 \text{ atmospheres,}$$

here outwardly directed, *i.e.*, against the direction in which the effective potential decreases. From the relations shown above, a possible resultant solution composition or activity ratio for KCl at 25° C may be computed. Thus,

$$2.303\,RT \log \frac{a_i}{a_e} = -NF\,(E)_{i-e}$$

$$56.3 \log \frac{a_i}{a_e} = 0.953$$

$$\frac{a_i}{a_e} = 1.04$$

Obviously, by inverse reasoning, movement of KCl between two phases with an effective solution composition ratio $\frac{a_i}{a_e}$ of 1.04, could set up a migration potential of inverse sign, across the boundary. The cause and effect relations at any time is a moot question.

Water:

It may be noted that all of the conditions which hold for solute would likewise hold for water, provided this analysis has any basis in fact.

Finally, it should be stated that the actual influences of galvanic effects are open to question as yet, in spite of the fact that much has been done in

the way of measurements of electrical potential differences across membranes and organs in biological materials.

Escaping tendency from curved surfaces

Since cellular and soil particle dimensions are small, and phasal surfaces may expose minute spacial irregularities, effective surface layers in nature may often be curved rather than planar. The escaping tendency in the limiting case here is a function of the size of the phase. Thus, the specific free energy of the components (solvent and solute) of solution or in a suspension is greater, the smaller the convex radius; and less, the smaller the concave radius of the phase. Two simple examples are given. First, within a given system, water will tend to migrate from a smaller drop to a larger body; second, adsorption on a substance depends not only on the amount of surface, but also largely on the degree of curvature of the particle and especially that of small cavities in the surface. Such an increase in specific free energy of both solute and solvent components of solution is related to an increase pressure within the phase (see above) caused by increased surface tension. Such influences, which may be quite negligible under relatively macro- conditions, become important where matter in a state of fine subdivision is dealt with (**13**, p. 125; **18**, pp. 251–253). The relationship for water may be expressed by the equation

$$F_1 = \frac{(\bar{f} - \bar{f}^\circ)}{\bar{v}} = \frac{2\gamma\bar{v}^\circ}{r\bar{v}^\circ} = \frac{2\gamma}{r} \qquad (12)$$

where γ is the surface tension and r is the radius. Such influences have not been evaluated for plant systems.

An analysis of a typical vacuolar solution may be made to judge the significance of the radius of curvature. The surface tension of water at 25° C is approximately 72 dynes per centimeter. The pressure normal to a plane water surface is therefore 72 dynes per square centimeter, or $72 \times 9.87 \times 10^{-7} = 7.10 \times 10^{-5}$ atmospheres. The figure 9.87×10^{-7} is the factor necessary for conversion from dynes per square centimeter to atmospheres. For a spheroid phase of radius 10^{-2} cm. (typical of a large vacuole in a larger cell):

$$F_1 = \frac{2\gamma}{r} = \frac{2 \times 72 \times 9.87 \times 10^{-7}}{10^{-2}} = 0.014 \text{ atmospheres.}$$

For a large vacuole in a smaller cell of radius 10^{-3} cm., $F_1 = 0.14$ atmospheres. The range for typically vacuolated cells is therefore of the order 0.014 – 0.14 atmospheres. It may be observed, therefore, that the effect of a spheroid aqueous body is not of significance generally. If the vacuolar sizes are of the order of colloidal dimensions (10^{-5} to 10^{-7} cm. radius) this effect could and may become very important. It may be suggested that such free energy differences of water between solution phases of colloidal

dimension in protoplasm of imperceptibly vacuolate cells may lead to the establishment of a single vacuole of macroscopic dimensions through migration of water from smaller to larger aqueous bodies of similar composition.

It may be noted from the foregoing discussion, that the specific free energy of a component in solution may be modified by the state of aggregation, *i.e.*, with relation to the radii of spheroid phases, within a system. Such is obviously not the case with plane surfaces. With the latter, change in the extent of surface only leads to a change in the total free energy of a component within a phase (**13**, pp. 124–125); **18**, pp. 247–248).

One further point should be made relative to recognition of an effect of surface tension on the specific free energy of the solvent. In an earlier work (**3**), two supplementary internal hydrostatic specific free energies were recognized, *viz.*, one termed intrinsic, the other extrinsic. The former was caused by a hydrostatic pressure inherently associated with the medium in the inner phase of the reference system, caused by restriction to expansion therein. Its effectiveness arose from a difference in osmotic specific free energy due to solute, between the two phases. It should be recognized that a part of this intrinsic energy intensity, though small and possibly insignificant in many cases, is related to the surface tension phenomena discussed above.

Influence of solutes, temperature, and pressure on surface tension

With reference to the above discussion, it may be pointed out that solutes in solution modify the surface tension of the mixture (related to a solute-solvent interaction coefficient) and thus the specific free energy of the components (**18**, pp. 249–251). Polar solutes generally increase while apolar substances in solution decrease the surface tension, varying within a homologous series. For purposes of penetrability discussion, arbitrary limits may be established. Thus some define non-electrolytes as all substances having no dissociation constants larger than 10^{-12}; strong electrolytes as all substances with one or more dissociation constants larger than 10^{-2}; weak electrolytes as those substances with dissociation constants between 10^{-2} and 10^{-12}. Different situations apply to the movement of substances in these first two classes, especially related to penetrability in the rate of net influx. For weak electrolytes, the situations are intermediate, approaching penetrabilities typical of either strong electrolytes or non-electrolytes, depending on the particular conditions, within limits characteristic of the species or organism.

Solute influences on the surface tension of a mixture are small, and provided the solution is not too dilute, it varies approximately in a linear manner with the logarithm of the concentration of the added substance. Temperature and pressure also alter the surface tension (**13**, pp. 152–156; **18**, pp. 247–249), but again these modifications are relatively small in magnitude.

Discussion

In the latter sections of this article, information has been gathered on the influences of various factors on the net flux intensities for solvent or solute between phases of a two-phased solution system. No indication has been given on, nor is it possible to predict in natural systems their influences on the proportionality coefficient of flux (**5**), or on the permeability coefficient (a property of the interposed interface or membrane, **5**). In actual cases, these factors lead to indeterminate theoretical evaluation of net rates of flux and/or energy expenditure. These can be evaluated only by experimental means.

One point further should be recognized, apart from the influences discussed above. In the over-all movement of materials with organisms, a steady state (not an equilibrium in the true sense of the term), of equal escaping tendency inward and outward for each constituent component of the system is approached with time. As the internal volume increases, other conditions being favorable, the composition of the sap of plants tends to remain relatively constant under usual conditions, through continued differential solute and water flux.

Summary

The escaping tendencies, or tendencies toward movement, of either solute or water between two solution phases of an osmometer should be recognized to be related to the differences of specific free energy or energy intensity, of the components. Should a visual viewpoint of this process for pedagogical purposes be desirable, a restricted molecular kinetic theory of pressure may be invoked. Where real pressures are considered, pressure of solution, in accord with hydrostatic pressures or turgor, only seem quantitatively reasonable; in all other kinetic pressure cases, the solvent viewpoint seems reasonable for water, and the solute viewpoint for solute of solutions, provided requisite limitations are recognized. In all cases, the first statement above is exact; all tendencies toward movement are expressed, completely and without limitation, in energy intensities (flux or diffusion intensities) of the constituent components.

A comparison is made of the relative importance of several factors which influence the flux of materials across a limiting membrane interposed in a two-phased system. Of these, solute concentration and pressure are of important magnitude to be generally considered. Temperature and surface factors, generally do not appear to be capable of exerting significant influences under natural conditions. Galvanic effects are difficult of evaluation and their importance is a moot question.

Division of Plant Nutrition
University of California
Berkeley, California

LITERATURE CITED

1. BRÖNSTED, J. N. Physical Chemistry. Chemical Pub. Co., New York. 1938.
2. BROYER, T. C. The movement of substances through a two-phased solution system. Science **105**: 67–69. 1947.
3. BROYER, T. C. The movement of materials into plants. I. Osmosis and the movement of water into plants. Bot. Rev. **13**: 1–58. 1947.
4. BROYER, T. C. The movement of materials into plants. II. The nature of solute movement into plants. Bot. Rev. **13**: 125–167. 1947.
5. BROYER, T. C. An outline of energetics in relation to the movement of materials through a two-phased solution system. Plant Physiol. **26**: 598–610. 1951.
6. BROYER, T. C. On the theoretical interpretation of turgor pressure. Plant Physiol. **25**: 135–139. 1950.
7. BROYER, T. C. A generalized comprehensive scheme for discussion of the movement of materials into living cells and tissues. Manuscript, 1950.
8. BROYER, T. C. Some gross correlations between growth enlargement and the solute and water relations of plants with special emphasis on the relation of turgor pressure to distention of cells. Plant Physiol. **25**: 420–432. 1950.
9. BURSTRÖM, H. A theoretical interpretation of the turgor pressure. Physiologia Plantarum **1**: 57–64. 1948.
10. CRAFTS, A. S., CURRIER, H. B., and STOCKING, C. R. Water in the Physiology of Plants. Chronica Botanica Co., Waltham, Massachusetts. 1949.
11. DEAN, L. A. and OVERSTREET, R. The availability of soil anions. In Monograph on Mineral Nutrition of Plants. University of Wisconsin Press, Madison. 1951.
12. EASTMAN, E. D. and ROLLEFSON, G. K. Physical Chemistry. McGraw-Hill Book Co., New York. 1947.
13. EDLEFSEN, N. E. and ANDERSON, A. B. C. Thermodynamics of soil moisture. Hilgardia **15**: 31–298. 1943.
14. EYSTER, H. C. Osmosis and osmotic pressure. Bot. Rev. **9**: 311–324. 1943.
15. FREY-WYSSLING, A. Physiology of cell wall growth. Ann. Rev. Plant Physiol. **1**: 169–182. 1950.
16. KRAMER, P. J. Plant and Soil Water Relationships. McGraw-Hill Book Co., New York. 1949.
17. KRAMER, P. J. and CURRIER, H. B. Water relations of plant cells and tissues. Ann. Rev. Plant Physiol. **1**: 265–284. 1950.
18. LEWIS, G. N. and RANDALL, M. Thermodynamics. McGraw-Hill Book Co., New York. 1923.

19. MARSHALL, C. E. Activity of cations adsorbed on soil colloids. In Monograph on Mineral Nutrition of Plants. University of Wisconsin Press, Madison. 1951.
20. MEYER, B. S. A critical evaluation of the terminology of diffusion phenomena. Plant Physiol. **20**: 142–164. 1945.
21. OLSON, A. R. and SIMONSON, T. R. Rates of ionic reactions in aqueous solutions. Jour. Chem. Phys. **17**: 1167–1173. 1949.
22. RICHARDS, L. A. and WEAVER, L. R. Moisture retention of some irrigated soils as related to soil-moisture tension. Jour. Agr. Res. **69**: 215–235. 1944.
23. RICHARDS, L. A. and WADLEIGH, C. H. Soil moisture and plant growth. Jour. Amer. Soc. Agron. In press. 1951.
24. URSPRUNG, A. Osmotic quantities of plant cells in given phases. (Translation by W. A. Beck.) Plant Physiol. **10**: 115–133. 1935.
25. WADLEIGH, C. H. and AYERS, A. D. Growth and biochemical composition of bean plants as conditioned by soil moisture tension and salt concentration. Plant Physiol. **20**: 106–132. 1945.
26. WASHBURN, E. W. Principles of Physical Chemistry. McGraw-Hill Book Co., New York. 1921.

BIOCHEMICAL COMPOSITION OF COTTON LEAVES AND THEIR CHEMICAL DEFOLIATION AS AFFECTED BY ENVIRONMENT[1]

VERNON L. HALL[2]

(WITH THREE FIGURES)

Received April 27, 1950

Induced defoliation of cotton became a necessity with the perfection of the mechanical cotton picker. Fortunately, a defoliant and some knowledge of its effectiveness was already available as a result of research to prevent boll rot in rank cotton (**7**). GULL (**4**) reported on the use of calcium cyanamide to remove the leaves from cotton plants to reduce the amount of leaf trash and stain in mechanically harvested cotton, and also, to reduce damage from certain insects and diseases that decrease production and lower the grade of lint cotton (**5**).

The initial field trials for induced defoliation were extremely successful and a solution to the problem was thought to have been found. In subsequent years, however, it was soon found that effective defoliation was dependent upon the status of environmental factors (**6**); the physiological condition of the leaf, at the time the defoliant is applied, becomes the determining factor in causing abscission.

Many field experiments have been conducted to determine the extent to which variation in different phases of the environment occasions alteration in the efficiency of chemical defoliation. From such studies it has been determined that vegetative-reproductive balance, moisture availability, and nutrient level each play a definite role in the efficiency of the defoliation process. EATON and RIGLER (**2**) report that a low concentration of carbohydrates in the cotton plant is responsible for much of the physiological shedding of small floral buds. Later, EATON and ERGLE (**1**) found that drought appeared to depress carbohydrate utilization to a greater extent than it did photosynthesis with the result that water deficit greatly increased the carbohydrate concentration within the cotton plant.

The following experiment was designed to determine the influence that variation in boll load, moisture availability, and nutrient supply would have upon defoliation and to investigate the associated biochemical status of the leaves. The study was carried out under greenhouse conditions so that other variables, besides those specifically controlled, could also be held relatively uniform. The investigation was designed as a factorial experiment in order to allow for statistical evaluation of each variable and its influence upon the other variables in the study.

[1] Research paper no. 936, Journal Series, University of Arkansas. Published with the permission of the Director of the Arkansas Agricultural Experiment Station.

[2] Present address: Botany Division, University of California, Davis, California.

Materials and methods

The plants were grown in soil using glazed two-gallon jars equipped with side arm drains fashioned after the type used by THARP (9) in studying fusarium wilt of cotton in nutrient sand cultures. Small gravel was used to prevent clogging of the drains, and a half-inch layer of washed sand was spread evenly over the bottom of each jar. A glass tube ten inches in length and 13 millimeters in diameter was used for subterranean irrigation. The tube was placed vertically in the jar opposite the drain. A glass fiber pad was used to prevent the watering tube from being forced to the bottom of the jar. When watering the plants, a funnel was inserted into the watering tube and a water-tight union was insured by the use of 1¼ inches of 5/16 × 1/16-inch rubber tubing. The rubber collar remained on the tube and the funnels could be easily removed.

The experiment involved 12 treatments incorporating two levels of soil fertility, two levels of water supply, and three variations in boll load. The variety of cotton selected was Deltapine 14; and the plants were grown, one per two-gallon jar, in four randomized blocks. Each treatment consisted of duplicate jars, (a) and (b), in order to obtain information on the effects of the variables upon both biochemical composition and defoliation.

The rich soil was made up of 50% compost, 17% sand, and 33% clay loam of low productivity. The poor soil contained 10% compost, 30% sand, and 60% clay loam soil. Nine kilograms of soil were used in each jar. Water levels were adjusted daily so that the plants given an adequate supply of moisture never wilted while the moisture-stressed plants were permitted to wilt slightly on alternate days. Flowers began to open when the plants were 55 days old. The fruiting load differentials were brought about by removing all open flowers from the "no boll-load" plots; alternate flowers from the "half boll-load" plots; and no flowers from the "full boll-load" plots. The plants with full boll-load, grown in rich soil, and receiving adequate moisture, stopped blooming when they were 87 days old. At this time the leaves were harvested from the plants in the (b) jars, and the leaves from the plants in blocks I and II were combined for one set of biochemical analysis and the leaves from the plants of blocks III and IV were pooled to make up a duplicate set of determinations.

Immediately following the harvest of leaves for the biochemical analysis, the defoliants were applied to all plants in the (a) jars as follows: Blocks I and III were sprayed with a 2% suspension of Aero Defoliant (60% calcium cyanamide) followed by six hours of simulated dew, and blocks II and IV were sprayed with a 1.88% solution of a liquid defoliant (20% free cyanamide) and then maintained in a relatively dry atmosphere. This method of treating alternate blocks with different defoliants has allowed for use of two types of defoliants. The Aero Defoliant requires the presence of moisture as dew for activation while the liquid defoliant is effective without the presence of dew. The difference between the number

of leaves on a plant at the time the defoliant was applied and ten days thereafter was calculated as the per cent. of defoliation.

The cotton leaf, consisting of the petiole and lamina, was used for the determination of carbohydrate and nitrogen fractions. The weight of green leaves was recorded for each individual plant and the leaves were dried until the weight at a temperature of 85° C became constant. Biochemical determinations were carried out for reducing sugars, non-reducing sugars, starches and dextrins, and hemicellulose in accordance with an outline of TOMPSETT (**10**), with the exception that the reducing reagents were those used by VAN DER PLANK (**11**), because of their greater stability. An error in the analytical procedure resulted in an erroneous value for the starch and dextrin content of the leaves from the plants grown in blocks I and II, grown in rich soil, under water stress, with no fruiting load. Due to the lack of sufficient material to re-analyze the sample, a substitute value was estimated using the missing plot technique of YATES (**13**), effecting an associated loss of one degree of freedom. The total nitrogen content of the leaves, including nitrates and nitrites, was determined by the method of LOOMIS and SHULL (**8**). The soluble nitrogen was determined by a procedure used by WADLEIGH (**12**), and the colloidal nitrogen was calculated as the difference between the total and the soluble nitrogen.

Results

A difference in effectiveness between the two types of defoliants was not established, neither as a net effect nor as an interrelation with other experimental factors. It is evident from table I, that the effectiveness of a defoliant is conditioned in a pronounced degree by the status of the plant as a result of variations in soil fertility, moisture availability, and fruit load. However, the data do not establish that these latter factors are interactive in conditioning a plant for chemical defoliation.

The evidence set forth in table II, suggests that the susceptibility to defoliation varies inversely with the amount of reducing sugar and the "starch and dextrin" in the leaves, while the non-reducing sugar and the hemicellulose content varies inconsistently with the per cent. of leaf drop. A statistical evaluation of the covariability between the per cent. of defoliation, and the various carbohydrate components revealed that the greatest degree of covariance was found between the starch and dextrin content of the leaves and the per cent. of defoliation. The coefficient of determination (r^2) of the total covariance for the latter relationship revealed that 61.31% of the variation in defoliation could be accounted for by the concomitant variation in the starch content of the leaves. The pooled regression for this relation (fig. 1) shows that for each 1% increase of starch in the leaves, the per cent. of defoliation decreased by 6.37% units. When the soil fertility levels are considered separately, a one percentile increase of starch in the leaves is associated with a decrease in defoliation of the plants grown

TABLE I

TYPE OF DEFOLIANT AND PER CENT. OF DEFOLIATION AT 10 DAYS

Soil fertility level	Soil water level	Boll load	Per cent. defoliation	
			Aero defoliant Blocks I and III Average	Liquid defoliant Blocks II and IV Average
Rich	Adequate	None	71.2	67.1
Rich	Adequate	Half	85.7	70.7
Rich	Adequate	Full	97.5	91.4
Rich	Stressed	None	53.7	58.6
Rich	Stressed	Half	64.7	63.9
Rich	Stressed	Full	73.3	71.0
Poor	Adequate	None	43.5	45.3
Poor	Adequate	Half	53.0	73.2
Poor	Adequate	Full	91.0	70.9
Poor	Stressed	None	28.7	12.9
Poor	Stressed	Half	34.1	5.0
Poor	Stressed	Full	51.7	54.2
	Average		62.3	57.0
	Standard error		4.77	3.24

NET EFFECT OF VARIOUS TREATMENTS ON MEAN PERCENTAGE OF DEFOLIATION

Soil fertility level	Moisture level		Fruiting load			Mean
	Adequate	Stressed	Full	Half	None	
Rich	80.6	64.2	83.3	71.3	62.7	72.4
Poor	62.8	31.1	67.0	41.3	32.6	47.0
Mean	71.7	47.7	75.2	56.3	47.6	59.7
			5%	1%		
	L.S.D.	2-level factor	9.35	12.51		
		3-level factor	11.64	15.33		

in the rich soil by 4.74 percentage units and 5.89 percentage units for those grown in the poor soil.

The rate of change in the per cent. of defoliation with the starch content of the leaves (fig. 2) is markedly different when the plants are grown with an adequate water supply than when they are grown under water stress. An increase of one percentile of starch in the leaves was accompanied by a decrease in defoliation of 8.50 percentage units for the plants grown with adequate water and 4.62 percentage units for the moisture-stressed plants. Each of the three fruiting loads in the study exhibited a distinct trend for the regressive influence of the starch content upon the per cent. of defoliation (fig. 3). For each one percentile increase in the starch content of the leaves there was a decrease of 5.25 percentage units in defoliation of the plants with no fruiting load; 13.99 for the plants with a half fruiting load, and 8.54 for those with a full fruiting load.

TABLE II

VARIATION IN PER CENT. DEFOLIATION AND PERCENTAGE BIOCHEMICAL COMPOSITION OF COTTON LEAVES AS AFFECTED BY VARIATION IN SOIL FERTILITY, SOIL MOISTURE SUPPLY, AND FRUITING LOAD. REPORTED AS PER CENT. DRY WEIGHT.

	Fertility level	Water level	Boll load	Average per cent. defoliation	Per cent. carbohydrate content of leaves				Per cent. nitrogen content of leaves		
					Reducing sugar	Non-reducing sugar	Starch and dextrin	Hemi-cellulose	Soluble nitrogen	Colloidal nitrogen	Total nitrogen
Blocks I and II	Rich	Adequate	None	67.9	1.94	0.34	3.34	5.27	0.84	2.63	3.47
	Rich	Adequate	Half	63.2	1.11	0.16	1.92	4.73	0.85	2.53	3.38
	Rich	Adequate	Full	91.5	1.23	0.38	1.51	4.32	0.91	2.38	3.29
	Rich	Stressed	None	59.2	2.42	0.60	8.26	3.97	0.74	1.75	2.49
	Rich	Stressed	Half	53.5	1.92	0.45	3.65	4.66	0.78	1.94	2.72
	Rich	Stressed	Full	63.7	1.27	0.38	3.31	3.71	0.85	2.26	3.11
	Poor	Adequate	None	43.6	1.37	0.46	7.48	4.74	0.45	2.08	2.53
	Poor	Adequate	Half	52.2	1.35	0.11	5.49	4.12	0.51	2.06	2.57
	Poor	Adequate	Full	74.2	0.69	0.15	3.51	4.93	0.62	2.10	2.72
	Poor	Stressed	None	23.3	2.12	0.21	12.80	4.68	0.61	1.75	2.36
	Poor	Stressed	Half	9.1	1.30	0.33	6.54	4.85	0.67	1.84	2.51
	Poor	Stressed	Full	55.6	1.37	0.14	6.82	4.66	0.67	1.60	2.27
Blocks III and IV	Rich	Adequate	None	70.4	1.27	0.28	3.69	5.26	0.69	2.38	3.07
	Rich	Adequate	Half	93.3	0.77	0.12	2.07	5.16	0.83	2.36	3.19
	Rich	Adequate	Full	97.5	0.56	0.14	1.71	4.72	0.79	2.58	3.37
	Rich	Stressed	None	53.2	2.24	0.79	7.69	4.53	0.74	1.73	2.47
	Rich	Stressed	Half	75.2	1.67	0.44	3.27	4.69	0.86	1.80	2.66
	Rich	Stressed	Full	80.7	1.42	0.55	2.56	4.77	0.80	1.70	2.50
	Poor	Adequate	None	45.3	1.24	0.53	6.22	5.02	0.54	2.07	2.61
	Poor	Adequate	Half	74.0	1.02	0.50	3.07	5.19	0.53	2.08	2.61
	Poor	Adequate	Full	87.8	0.84	0.27	2.99	4.86	0.57	2.22	2.79
	Poor	Stressed	None	18.4	2.24	0.59	10.70	4.68	0.68	1.58	2.26
	Poor	Stressed	Half	30.0	1.46	0.44	4.93	4.54	0.72	1.74	2.46
	Poor	Stressed	Full	50.4	1.26	0.38	4.86	4.72	0.75	1.81	2.56

Analysis of covariance revealed that there was a high degree of covariability for starch and defoliation between fertility levels, between soil moisture regimes, and between fruiting conditions; but the residual covariance between these two entities was of no consequence. A multiple correlation analysis (**3**) was carried out between defoliation as the dependent variable

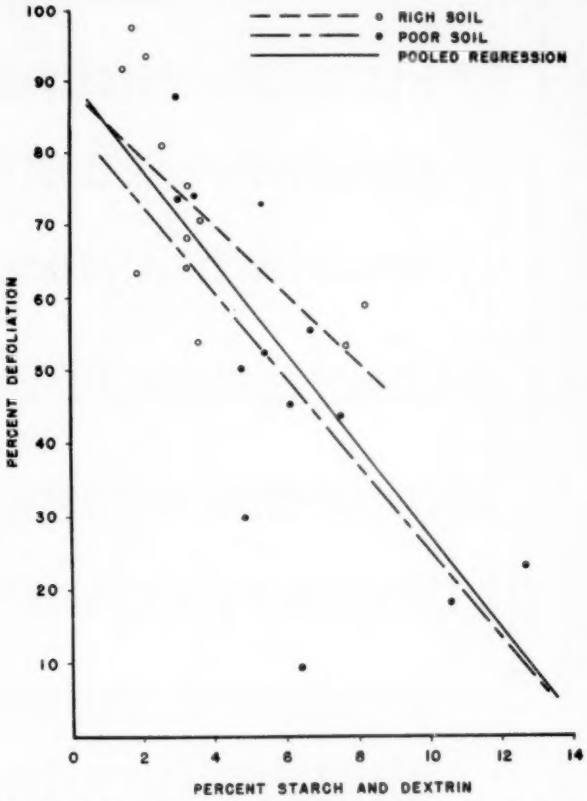


FIG. 1. The influence of soil fertility levels upon defoliation and the starch and dextrin content of cotton leaves.

and the starch, reducing sugar and non-reducing sugar content of the leaves. The resulting R^2 value was virtually no larger than the one obtained for the simple correlation, r^2 (61.31%), between defoliation and starch. This emphasizes that starch content was the best predictor of the carbohydrate fractions studied for assessing susceptibility of cotton plants to chemical defoliation.

Correlation analysis revealed that the amount of defoliation obtained in this study was in direct relation to the total nitrogen content of the leaves (table II), and that there was a better relation between total nitrogen and defoliation than between defoliation and each of the components of total nitrogen. The study suggests that the variation in defoliation may be

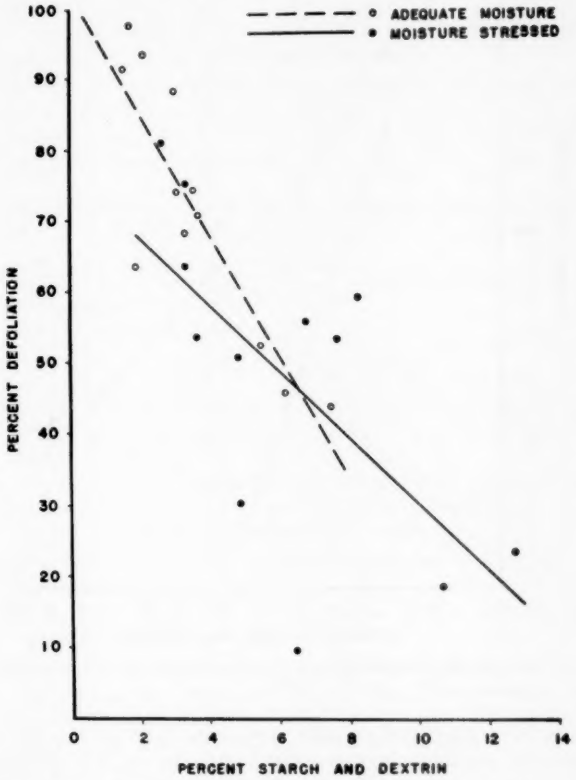


FIG. 2. The influence of soil moisture supply upon defoliation and the starch and dextrin content of cotton leaves.

more accurately accounted for if the effects of both total nitrogen and starch are taken into consideration; however, multiple correlation failed to account for a greater proportion of variance in defoliation over that from the simple relation with starch alone. Also, there was no improvement in accounting for variance in defoliation by taking into consideration starch plus colloidal or starch plus soluble nitrogen. Thus, it was revealed that although there

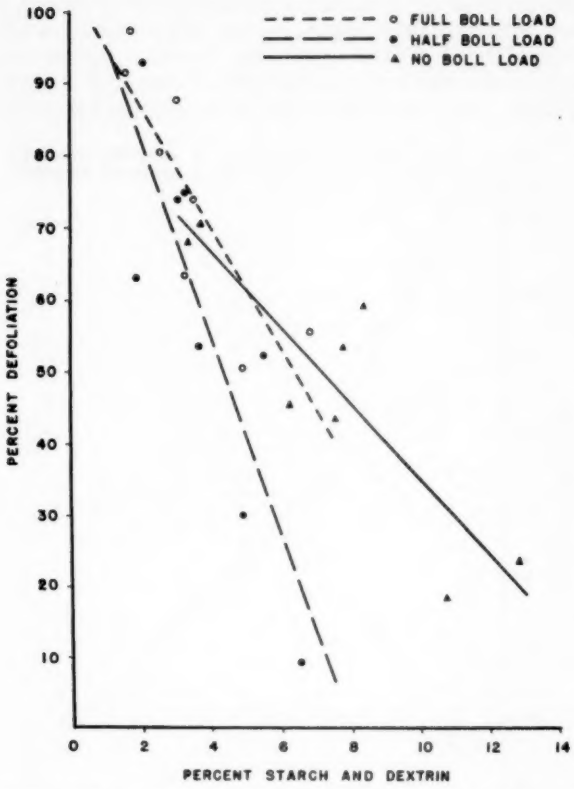


FIG. 3. The influence of fruiting load upon defoliation and the starch and dextrin content of cotton leaves.

appears to be a significant positive correlation between the per cent. of defoliation and the total nitrogen content of the leaves, this effect was due to inter-covariance between starch and total nitrogen.

Summary

This study has shown that the various environmental conditions brought to bear upon cotton plants induced marked changes in the degree of defoliation as a result of treating with chemical defoliants. There is a high degree of negative covariance between sensitivity to defoliation and the starch content of the leaves. This inverse relationship between defoliation and the starch content of the leaves suggests that carbohydrate depletion is an important factor in the effectiveness of chemical defoliation.

Although there appeared to be a significant positive effect between defoliation and the total nitrogen content of the leaves it was found that the influence of nitrogen upon defoliation was largely due to an interrelation between starch and total nitrogen. A simultaneous consideration of starch and total nitrogen in evaluating the degree of defoliation gave no improvement in precision over consideration of starch alone.

The study has revealed that it may be possible to predict, with fair accuracy, the susceptibility of a cotton field to chemical defoliation by making an assay of the starch content of the plants.

The marked diversion between starch content and defoliation within the categories of soil fertility, moisture supply and crop load indicate that there are factors other than starch content which determine the susceptibility of a cotton plant to chemical defoliants.

The writer wishes to acknowledge the advice and assistance of Dr. W. H. Tharp and Dr. C. H. Wadleigh.

UNITED STATES DEPARTMENT OF AGRICULTURE
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
UNIVERSITY OF ARKANSAS
FAYETTEVILLE, ARKANSAS

LITERATURE CITED

1. EATON, F. M. and ERGLE, D. R. Carbohydrate accumulation in the cotton plant at low moisture levels. Plant Physiol. **23**: 169–185. 1948.
2. EATON, F. M. and RIGLER, N. E. Effect of light intensity, nitrogen supply, and fruiting on carbohydrate utilization by the cotton plant. Plant Physiol. **20**: 380–411. 1945.
3. EZEKIEL, M. Methods of Correlation Analysis. 1st ed., pp. 136–186. Wiley, New York. 1930.
4. GULL, P. W. Cotton defoliation. Mississippi Delta Branch Expt. Sta. Serv. Sheet no. 364. 1943.
5. GULL, P. W. Cotton defoliation. Suppl. to Mississippi Delta Branch Expt. Sta. Serv. Sheet no. 364. Mimeo. Rpt. 1944.
6. GULL, P. W. and DUNNAM, E. W. Experiments in chemical cotton defoliation. Repr. from Agr. Chemicals. June, 1947.
7. HALL, E. E. and HARRELL, F. M. Defoliating cotton with cyanamid. 55th Ann. Rpt., South Carolina Agr. Expt. Sta., pp. 103–104. 1942.
8. LOOMIS, W. E. and SHULL, C. A. Methods in Plant Physiology. 1st ed., pp. 309–312. McGraw-Hill Book Co., Inc., New York. 1937.
9. THARP, W. H. A sand-nutrient infection technique for the study of Fusarium wilt of cotton. Phytopath. **28**: 206–209. 1938.
10. TOMPSETT, S. L. Critical analysis of the reduction of alkaline copper reagents by glucose and other substances. Biochem. Jour. **24**: 1148–1163. 1930.

11. VAN DER PLANK, J. E. The estimation of sugars in the leaf of the Mangold (*Beta vulgaris*). Biochem. Jour. **30**: 457–483. 1936.
12. WADLEIGH, C. H. Growth status of the cotton plant as influenced by the supply of nitrogen. Arkansas Agr. Expt. Sta. Bull. 446. 1944.
13. YATES, F. The analysis of replicated experiments when the field results are incomplete. Empire Jour. of Expt. Agr. **1**: 129–142. 1933.

DIFFERENCE IN ACTIVITY BETWEEN 2,4-DICHLOROPHENOXYACETIC ACID AND OTHER AUXINS, AND ITS SIGNIFICANCE IN HERBICIDAL ACTION

J. VAN OVERBEEK, RENÉ BLONDEAU AND VESTA HORNE

(WITH THREE FIGURES)

Received November 7, 1950

Introduction

Many reports are available in the literature on the effects of 2,4-D on numerous physiological and biochemical processes. Among these are increased cell proliferation (**5**), increased respiration (**20**), increased carbohydrate depletion (**17**), decreased respiration of roots (**27**), decreased uptake of potassium by roots (**18**), decreased accumulation by the root system of KNO_3 and KCl (**13**), inhibition of lipase activity (**9**), inhibition of ascorbic acid oxidase (**27**), stimulation of phosphatase activity (**15**), stimulation of beta-amylase activity (**22, 28**), inhibition of alpha- and beta-amylase activity (**14**), etc. Useful though these observations are for the understanding of auxin action, they do not tell us if these physiological changes are the specific reasons 2,4-D has such a high herbicidal activity. It would rather seem that these phenomena are not specific for 2,4-D but are caused as well by other auxins such as indoleacetic acid and naphthaleneacetic acid (for terminology see **22**). Thus, an increased respiration, a stimulated proliferation, and a considerable loss of carbohydrate are caused in the Jerusalem artichoke tuber by indoleacetic acid (**8**). Increased phosphatase activity is found in Avena roots after treatment with indoleacetic acid and other auxins (**4**). Inhibition of amylase activity by indoleacetic acid and by a variety of other auxins has been demonstrated (**26**). Indoleacetic acid, though capable of affecting many reactions in a fashion similar to 2,4-D is definitely not an efficient herbicide.

The clue to the solution of why 2,4-D displays such a high herbicidal activity appears to lie in a different direction. Rather than only asking: "What physiological changes does 2,4-D bring about?" it would appear to be more profitable to ask first: "In what fundamental aspect does 2,4-D differ from the other auxins?" The present paper will largely deal with this question.

Materials and methods

Etiolated pea stems of the Alaska variety constituted the plant material. The growing regions of these stems were used either in the section test or in the pea test. Details of these tests may be found elsewhere (**21, 22, 24**). During the testing period, which lasted 16 hours, the cut pea stems remained in 0.05 or 0.01 M phosphate buffer solutions. The higher concentration was

used in earlier experiments, the lower concentration in most later tests. The latter concentration was shown by AUDUS (**1**) to give the most satisfactory results also. To the buffer solutions were added 1% sucrose and auxin of the desired type and quantity. Two pH values were used: pH 5.5 and pH 6.2. These were chosen because the pH of the cytoplasm of plants falls within this range (**19**). It is generally agreed at present that the cytoplasm is the site of auxin activity.

In the section tests seven sections, initially 5.4 mm. in length, were bathed in 2 cc. of solution per 50-cc. beaker on a shaker. In the pea test five split stems were in 30 cc. of solution per 4½-inch stacking dish. The beakers and dishes were sterilized. It was found that this simple expedient was sufficient to keep bacterial contamination of the test solutions to a minimum. Without any aseptic precautions, solutions were found to become cloudy during the test; in such contaminated dishes a reduced auxin reaction was observed. During the testing period, the pea stems remained in darkness at 81 ± 1° F. Each test was run in triplicate and in addition repeated at least once on a different day. The standard error of the mean was calculated, the range of which is indicated in the graphs by the small vertical lines.

The dissociation constant of each auxin was determined by dissolving the substance in 100 cc. of CO_2-free water with the aid of 1 cc. of isopropyl alcohol to give a 0.001 M solution. The pH of this solution was measured rapidly with the glass electrode and the K and pK calculated according to standard procedure (see for instance **16**). The results are summarized in table I, in which the concentrations of undissociated auxin molecules are

TABLE I

DISSOCIATION DATA OF SIX AUXINS ARRANGED IN ORDER OF INCREASING DISSOCIATION

	K	pK	[HA] in 10^{-3} M and pH 6.2	[HA] in 10^{-3}M and pH 5.5
Indoleacetic acid	2.8×10^{-5}	4.55	2.2×10^{-5}	1.03×10^{-4}
Alpha-naphthaleneacetic acid	6.17×10^{-5}	4.21	1.0×10^{-5}	4.9×10^{-5}
Cis-cinnamic acid	1.1×10^{-4}	3.96	5.7×10^{-6}	2.85×10^{-5}
Beta-naphthoxyacetic acid	1.25×10^{-3}	2.89	5.5×10^{-7}	2.75×10^{-6}
2,4-D	1.55×10^{-3}	2.81	4.06×10^{-7}	2.03×10^{-6}
2,4,5-T	2.66×10^{-3}	2.57	2.38×10^{-7}	1.18×10^{-6}

given at the two pH values employed in these tests. The pK values found in the literature have been reported elsewhere (**24**).

The auxins investigated were: indoleacetic acid (IA), now widely recognized as a native auxin, alpha-naphthaleneacetic acid (NA), beta-naphthoxyacetic acid (NO), cis-cinnamic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). 2,4-D and 2,4,5-T are highly effective herbicides. The 2,4,5-T and NO were tested only in the

split pea stem test, while the remaining auxins were tested in both the split pea stem test and the pea stem section test.

Results

When auxin activity in the pea test is plotted against auxin concentration the broken lines of figure 1 are obtained. Evidence has accumulated (**1, 3, 13**) to the effect that auxin activity does not so much depend upon the total auxin concentration, as upon the concentration of undissociated

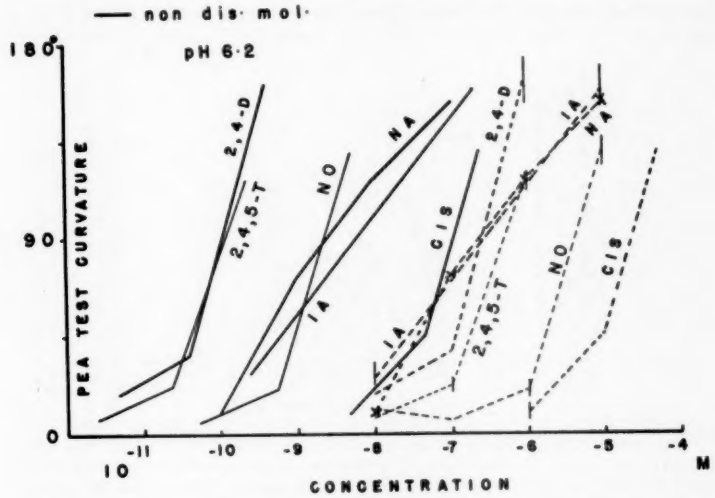


FIG. 1. Curvatures of six different auxins in the pea test as a function of the auxin concentration. Curvature in the buffer solution alone is rated 0. Broken lines plotted on the basis of molar concentration not corrected for dissociation. Solid lines plotted on the basis of undissociated auxin molecules. The short vertical lines indicate the range of the standard error of the mean. 2,4-D = 2,4-dichlorophenoxyacetic acid; 2,4,5-T = trichlorophenoxyacetic acid; NO = beta-naphthoxyacetic acid; NA = alpha-naphthaleneacetic acid; IA = indolcacetic acid; CIS = cinnamic acid.

molecules. Consequently, with the aid of the data of table I auxin activity was plotted in terms of undissociated molecules. The results are given as the solid lines in figure 1.

In order to make certain that the results obtained were not due to some inherent characteristics of the pea test, experiments with four auxins were repeated using pea stem sections for testing material. The results of these tests are shown in figure 2. A comparison between the results obtained from curvatures of split pea stems (fig. 1) and those from elongation of isolated sections of the pea stem shows indeed that the curves for the various auxins are almost identical for the two tests. Both the position of the curves as

well as their slopes are identical regardless of test method. An exception is indoleacetic acid, which has a curve which is considerably steeper in the pea test than in the section test.

The data of figures 1 and 2 were compiled from averages from individual tests extending over a period of several months. In order to ascertain that the difference in slope of the IA curves is real, IA and 2,4-D were tested simultaneously in both pea and section tests. The results obtained (fig. 3) confirm the conclusions drawn from a comparison between figures 1 and 2. The shape of the concentration curve of IA is indeed dependent upon the type of test; the 2,4-D concentration curve is independent of the type of test.

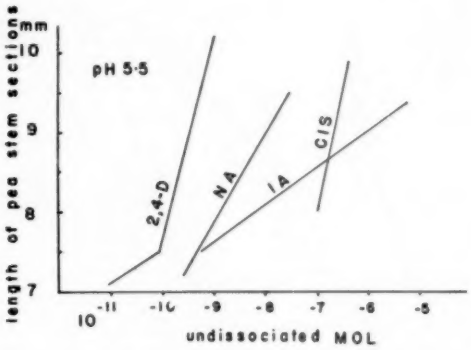


FIG. 2. Increase in straight growth of pea stem sections by four different auxins as a function of the concentrations of undissociated auxin molecules. Initial section length 5.4 mm.; duration of test, 16 hours. The abscissa is drawn at the growth level occurring in the buffer alone. Standard error of the mean, 0.1 mm. or less.

Further analysis showed that the difference in IA concentration curve between the split pea and the stem section test is not due to the small volume of solution employed in the section test, as variation of this factor did not materially affect the slope of the curve. The effect is probably due to the nature of the pea test in which the curvature of the stem halves results from a difference in elongation between the inside and the outside portions of the stems. As was already pointed out at an earlier date (**25**), low concentrations of indoleacetic acid cause considerable growth of both the inside and the outside of the stem halves, thereby concealing a growth effect of IA at low concentrations. This effect results in a concentration curve for the pea test which appears steeper than it really should be on the basis of promotion of straight growth (fig. 3 A). The section test, therefore, is a more reliable test for elongation effects than the pea test.

From the concentration curves of figures 1 and 2 the following conclusions may be drawn: The activity of the various auxins differs greatly. The

most active of the auxins tested are 2,4-D and 2,4,5-T. The slopes of the concentration curves differ greatly. 2,4-D and cis-cinnamic acid have steep slopes, while naphthaleneacetic acid and indoleacetic acid have flatter lines. The significance of these two points will be discussed below.

Discussion

From the original data of ZIMMERMAN (**29**) one might conclude that 2,4-D is not more active than IA or NA, as all three of these compounds have the concentration of 0.0015% as the lower limit for activity in causing an auxin curvature in the tomato petiole. Indeed, this agrees with the dotted curves (data not corrected for dissociation) of figure 1. However,

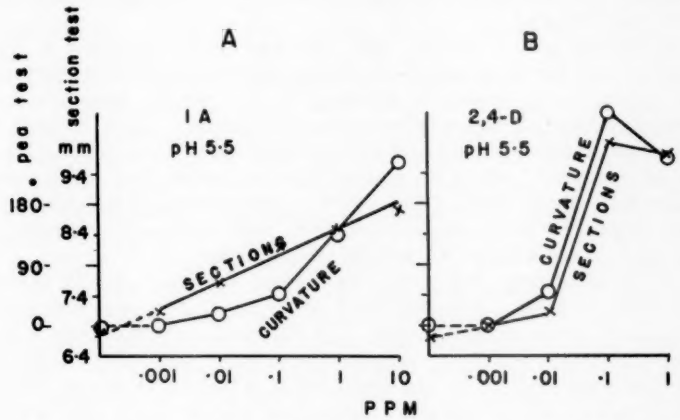


FIG. 3. Direct comparison of section and pea tests. A. With indoleacetic acid. Averages of three tests each run in triplicate. B. With 2,4-D. Averages of one test run in triplicate. In low concentrations of IA the pea test curvature lags behind the growth effect as measured by the section test.

if the concentration/activity curves are corrected for dissociation (solid curves of figures 1 and 2) it becomes at once apparent that the activity of 2,4-D is higher than that of IA or NA. At no point does the 2,4-D curve for undissociated molecules cross any other auxin curve. The compound 2,4,5-T is considered here together with 2,4-D, having a similar activity in the pea test. Over its entire range, therefore, 2,4-D is more active than any of the other auxins. This fact clearly invalidates D. M. Bonner's earlier thesis (**3**) that on the basis of undissociated molecules all auxins have the same activity. The untenability of this thesis was also discussed by AUDUS (**1**).

In another publication (**24**) it was pointed out that 2,4-D exceeded other auxins in balancing the anti-auxin effect of a given amount of trans-cinnamic acid. There it was shown that one undissociated molecule of 2,4-D had

an effect equivalent to 10 of NA, 100 of cis-cinnamic acid, and 500 of IA. It was concluded that this fact might reflect the affinity of these auxins for the cytoplasmic protein with which they are thought to combine. In its greater activity, undissociated molecule for undissociated molecule, one has one point in which 2,4-D differs from the other auxins.

The importance of a high auxin activity for success as a herbicide is also illustrated for beta-naphthoxyacetic acid (NO). In the activity diagram of figure 1 its position is well to the right of that of 2,4-D. For equal auxin effects at least 10 times higher NO than 2,4-D concentrations are required. This fact explains McNew and Hoffmann's evaluation (**12**): "Although the beta-naphthoxyacetic acid is a recognized growth regulant, with strong ability to induce formative effects, it does not operate effectively as a herbicide at low dosage."

If the solid lines of figures 1 and 2 are once more compared, it will be observed that due to diverging auxin curves differences in activity between the auxins are not necessarily constant, but that between certain auxins these differences increase with increasing concentration. Thus, in figure 2 a comparison between the 2,4-D and NA curves shows that to produce a section length of 7.5 mm. a NA concentration is required which is five times higher than that of 2,4-D. To produce a section length of 8.5 mm. a 10 times higher NA concentration is required, while for a section length of 9.5 mm. a 15 times higher NA concentration is needed.

In the same figure 2 a comparison between the 2,4-D and IA curves shows that to produce a section length of 7.5 mm. a five times higher IA than 2,4-D concentration is needed. For this small auxin effect this is the same difference in activity as was found between NA and 2,4-D. In order to increase the length of the pea stem sections to 8.5 mm. it requires an IA concentration which is 130 times higher than that of 2,4-D. For a still greater auxin effect, a growth promotion to 9.5 mm., a comparison of the curves will now show that it takes an IA concentration which is 10,000 times larger than that of 2,4-D. In other words, one undissociated molecule of 2,4-D will do the work of 10,000 undissociated molecules of the native auxin indoleacetic acid.

In figures 1 and 2 in which the ordinate reads pea test curvature and length of stem sections, the curves may not be extrapolated further upward because of the well known optimum curve expressing the relation between auxin effect and auxin concentration (**22**, p. 440). However, when the ordinate is made to read "auxin effect" instead of "length of sections" or "pea test curvature," it would seem allowable to extrapolate the 2,4-D and IA curves upward. When this is done, it will be clear that when auxin effects are reached which are herbicidal, it will take 100,000 to 1,000,000 or perhaps even more molecules of undissociated IA to do the work of only one undissociated 2,4-D molecule. For NA intermediate figures may be expected. This effect of the diverging auxin curves is another aspect in which 2,4-D

differs from the other auxins, and may well constitute another reason for its great activity as a herbicide.

Having established two principal physiological differences between 2,4-D and other auxins, one might next ask how auxins of high activity and in high concentration bring about the death of a plant. The literature quoted in the introduction, as well as many additional papers, is a clear indication that under the influence of auxins the metabolism of the plant changes.

Evidence now on hand suggests that the reason for the lethal action of 2,4-D may be found in this changed metabolism. It is well possible that metabolites which normally are produced in small harmless quantities are produced in the changed metabolism in larger, toxic quantities. FULTS and JOHNSON (**6**) have demonstrated this with scopoletin. This compound, a coumarin derivative, is normally present in small quantities (see also **7**). Under the influence of 2,4-D much larger quantities accumulate. Coumarin and many of its derivatives are phytotoxic and thus Fults and Johnson suggested that the increased concentration of scopoletin is the direct cause of the phytotoxic action of 2,4-D. This way of thinking makes understandable the earlier observations by AUDUS and QUASTEL (**2**) who studied the effect of coumarin: "With respect to the inhibition of root-growth, one feature of the activity of coumarin is its broad similarity to the action of 2,4-dichlorophenoxyacetic acid."

Reasoning along similar lines has also yielded a clue for the selective action of 2,4-D. It is well established that 2,4-D is a selective herbicide which affects broad-leaved plants more than grasses. How this selective action is brought about has long puzzled plant physiologists. It has recently become known that beta-methyl umbelliferone, another derivative of coumarin, is more phytotoxic to broad-leaved plants than to grasses (**10**). Umbelliferone, like scopoletin, is a normal plant metabolite. Both are demonstrable in the plant by their fluorescence in UV radiation. These facts suggest that accumulation of coumarin derivatives under the influence of 2,4-D might well explain the selective herbicidal effect of this auxin.

Another aspect also may be considered here briefly. In an earlier publication (**24**) it was shown that trans-cinnamic acid, also a naturally occurring coumarin derivative, is an anti-auxin. In relatively low concentrations such as 15 p.p.m. the anti-auxin effect of this compound could be completely reversed by auxins. However, in higher concentrations such as 30 p.p.m., it was noticed that trans-cinnamic acid displayed symptoms of phytotoxicity, but only in the presence of relatively high concentrations of auxins (such as 10 p.p.m. of naphthaleneacetic acid). It therefore appears as if such relatively high auxin concentrations make the tissue of the plant more susceptible to damage by naturally occurring metabolites. This also is probably another aspect of the same general phenomenon, the drastic change in the plant's normal metabolism under influence of auxins.

Summary

The herbicidal action of 2,4-D is considered to consist of two steps: (1) the very high activity of 2,4-D as an auxin, and (2) the metabolic changes brought about by this auxin activity. It is in the first step that 2,4-D differs from the other, "non-herbicidal," auxins. It is a quantitative rather than a qualitative difference. It is in the second step that the phytocidal effects take place.

The differences between 2,4-D and "non-herbicidal" auxins are on the basis of undissociated molecules: (1) a considerably greater auxin activity at all concentrations, and (2) an increasingly greater relative activity with increasing auxin concentration. Thus in the pea stem section test an increase in length of about 10% over that of the control is brought about by a concentration of undissociated molecules of indoleacetic acid which is about five times higher than that of 2,4-D. But in order to bring about a 35% increase in the length of the stem sections it takes a concentration of IA which is 10,000 times greater than that of 2,4-D. With this in mind, it is conceivable that in the range of herbicidal effects one undissociated molecule of 2,4-D may have the activity equivalent to 1 million or more undissociated molecules of indoleacetic acid.

Based on data available in the literature it was reasoned that auxins bring about a change in the plant's metabolism. Abnormally high auxin concentrations might conceivably lead to abnormal accumulations of metabolites, such as coumarin derivatives. Because of its very high auxin activity 2,4-D can be expected to bring about such metabolic changes to a larger degree than other, "non-herbicidal" auxins. One of these coumarin derivatives, β-methyl umbelliferone, has been shown to be more toxic to broad-leaved plants than to grasses. Thus, a 2,4-D-induced accumulation of metabolites, among which coumarin derivatives suggest themselves at the present state of knowledge, is capable of explaining both the phytotoxicity and the selectivity of this auxin herbicide.

The assistance of Dr. E. F. Feichtmeir in the determination of the dissociation constants is gratefully acknowledged.

SHELL OIL COMPANY
AGRICULTURAL LABORATORY
MODESTO, CALIFORNIA

LITERATURE CITED

1. AUDUS, L. J. Studies on the pH-relationships of root growth and its inhibition by 2,4-dichlorophenoxyacetic acid and coumarin. New Phytol. **48**: 97–114. 1948.
2. AUDUS, L. J. and QUASTEL, J. H. Coumarin as a selective phytocidal agent. Nature **159**: 320–324. 1947.
3. BONNER, D. M. Relation of environment and of the physiological properties of synthetic growth substances to the growth reaction. Bot. Gaz. **100**: 200–214. 1938.

4. BONNER, J. Personal communication. Pacific Coast Meetings A.A.A.S. 1950.
5. BURTON, D. F. Formative effects of certain substituted chlorophenoxy compounds on bean leaves. Bot. Gaz. **109**: 183–194. 1947.
6. FULTS, J. and JOHNSON, M. A cumulative fluorescent chemical found in certain plants treated with 2,4-D identified as scopoletin. Abst., West. Weed Conf. Denver. January, 1950.
7. GOODWIN, R. H. and TAVES, C. The effect of coumarin derivatives on the growth of Avena roots. Amer. Jour. Bot. **37**: 224–231. 1950.
8. GORIS, A. Exhaustion of reserve sugars in pieces of Jerusalem artichoke tubers grown *in vitro* on a sugarless medium: influence of 3-indoleacetic acid. Compt. Rend. **226**: 742–744. 1948.
9. HAGEN, C. E., CLAGETT, C. O., and HELGESON, E. A. 2-4-Dichlorophenoxyacetic acid inhibition of castor bean lipase. Science **110**: 116–117. 1949.
10. HAMNER, C. L. and SELL, H. M. Selective inhibitory action of beta-methyl umbelliferone. Amer. Soc. Hort. Sci. Meetings. Columbus, Ohio. September 12, 1950.
11. MITCHELL, J. E., BURRIS, R. H., and RIKER, A. J. Inhibition of respiration in plant tissues by callus stimulating substances and related chemicals. Amer. Jour. Bot. **36**: 368–378. 1949.
12. MCNEW, G. L. and HOFFMAN, O. L. The growth-regulant, herbicidal and physical properties of 2,4-D and related compounds. Iowa State Coll. Jour. of Sci. **24**: 189–208. 1950.
13. NANCE, J. F. Inhibition of salt accumulation in excised wheat roots by 2,4-dichlorophenoxyacetic acid. Science **109**: 174–176. 1949.
14. NEELY, W. B., BALL, C. D., HAMNER, C. L., and SELL, H. M. Effect of 2,4-dichlorophenoxyacetic acid on the alpha and beta amylase activity in the stems and leaves of red kidney bean plants. Science **111**: 118. 1950.
15. OLSEN, K. L. Effect of 2,4-D on the phosphatase enzyme in *Zea mays* and *Vigna sinensis* seedlings. Amer. Soc. Plant Physiol. West. Sec. Salt Lake City meetings. June 23, 1950.
16. PAULING, L. General Chemistry. Freeman and Co., San Francisco. 1947.
17. RASMUSSEN, L. W. The physiological action of 2,4-dichlorophenoxyacetic acid on dandelion. Plant Physiol. **22**: 377–392. 1947.
18. RHODES, A. The effect of the plant growth regulator 4-chloro-2-methyl phenoxyacetic acid on the mineral and nitrogen content of plants. Absts. 2nd Internatl. Cong. of Crop Protection. Section **3**: 32–33. 1949.
19. SMALL, J. pH and Plants. D. Van Nostrand Co., New York. 1946.
20. SMITH, F. G., HAMNER, C. L., and CARLSON, R. F. Changes in food reserves and respiratory capacity of bindweed tissues accompanying herbicidal action of 2,4-dichlorophenoxyacetic acid. Plant Physiol. **22**: 58–65. 1947.

21. THIMANN, K. V. Plant growth hormones. In G. Pincus and K. V. Thimann, The Hormones. Academic Press, New York. **1**: 5–119. 1948.
22. VAN OVERBEEK, J. Plant hormones and other regulatory factors. In D. E. H. Frear, Agricultural Chemistry I. D. Van Nostrand Co., New York. **1**: 422–463. 1950.
23. VAN OVERBEEK, J. Use of synthetic auxins as herbicides in the tropics. In H. B. Tuckey, Plant Hormones in Agriculture. John Wiley and Sons, New York. (In press.)
24. VAN OVERBEEK, J., BLONDEAU, R., and HORNE, V. Trans-cinnamic acid as an anti-auxin. Amer. Jour. Bot. (In press.)
25. VAN OVERBEEK, J. and WENT, F. W. Mechanism and quantitative application of the pea test. Bot. Gaz. **99**: 22–41. 1937.
26. VOLKER, J. F. Compounds capable of plant amylase inhibition. Science **112**: 61. 1950.
27. WEST, F. R. and HENDERSON, J. H. M. The effect of 2,4-dichlorophenoxyacetic acid and various other substances upon the respiration of blue lupin seedling roots. Science **111**: 579–581. 1950.
28. WORT, D. J. The effect of non-herbicidal concentrations of 2,4-D on the composition of buckwheat. Amer. Soc. Plant Physiol. West. Sec. Salt Lake City meetings. June 23, 1950.
29. ZIMMERMAN, P. W. Formative influences of growth substances on plants. Cold Spring Harbor Symposia **10**: 152–157. 1942.

ENZYMATIC CONVERSION OF INDOLE ACETALDEHYDE AND NAPHTHALENE ACETALDEHYDE TO AUXINS

POUL LARSEN

(WITH TWO FIGURES)

Received February 2, 1951

Introduction

A neutral substance, convertible to an acid auxin, is present in various plant extracts (**5, 9**) and chemical preparations (**9**). As indicated by a number of biological tests, the neutral substance is in all likelihood 3-indole acetaldehyde and will for convenience be designated by this name in the present paper.

In a previous paper (**11**) it was shown that 3-indole acetaldehyde is rapidly converted to indoleacetic acid in excised Avena coleoptiles and in fresh, but not in boiled, coleoptile juice. It was concluded that the conversion is caused by an enzyme system present in the coleoptiles. The indole acetaldehyde used in those experiments was prepared from tryptophan and isatin using a method described previously (**9**, p. 85).

GORDON and NIEVA (**5, 6**), using indole acetaldehyde extracted from pineapple leaves and purified via the bisulphite addition-product, demonstrated the presence of an enzyme system, capable of converting indole acetaldehyde to indoleacetic acid, in breis and protein preparations obtained from pineapple leaf bases. WILDMAN *et al.* (**17**) failed to obtain any conversion of indole acetaldehyde to indoleacetic acid by enzyme preparations from spinach leaves, although such preparations produced indoleacetic acid readily from tryptophan. As already pointed out by GORDON and NIEVA (**6**), however, the synthetic preparation of indole acetaldehyde used by WILDMAN *et al.* (**17**) probably contained very little, if any, of the desired aldehyde.

Since indole acetaldehyde seems to be the immediate precursor of indoleacetic acid in the biosynthesis of this auxin, a method for the determination of indole acetaldehyde in plant extracts would be highly desirable. This aldehyde, however, has not yet been prepared in the chemically pure state; so we are unable to construct a standard activity curve, relating biological effect, such as curvature in the Avena test, to actual concentration of indole acetaldehyde. Amounts of indole acetaldehyde have been expressed in terms of amounts of indoleacetic acid obtained after treatment of the preparation or extract with soil (**5, 6, 9, 10, 15**). Owing to losses occurring during the treatment this procedure is unsatisfactory. Since indole acetaldehyde does produce curvatures in the Avena test these curvatures can be compared with curvatures produced by pure indoleacetic acid. As an arbitrary, biological unit of indole acetaldehyde was proposed the γ-"equiva-

lent" (**11**), defined as the amount of aldehyde which has the same effect in the Avena test as one γ of indoleacetic acid when tested at such a dilution that the resulting curvatures are $\leqq 15°$ (or with water-cultured test plants $\leqq 5°$).

In the previous study of enzymatic conversion (**11**), it was found that treatment of one γ-"equivalent" of indole acetaldehyde with coleoptile juice gave rise to the formation of approximately 5 γ of indoleacetic acid. (Indoleacetic acid is not inactivated by coleoptile juice.) This means that one γ-"equivalent" of the aldehyde weighs at least about 5 γ, but since it is unknown what losses of aldehyde may have occurred the 5 γ is a minimum value. The main purpose of the present study was to estimate the absolute weight of one γ-"equivalent" of indole acetaldehyde through a determination of the amount of acid growth substance which can be recovered from a known amount of aldehyde after treatment with coleoptile juice. Because pure indole acetaldehyde is still unavailable, crystalline naphthalene acetaldehyde was used as a model substance. Naphthalene acetaldehyde has been shown previously to be active in the Avena test (**10**), the pea test (**14**, p. 298), and a root-inhibition test (**1**). ASHBY (**1**), working in this laboratory, showed that juice expressed from roots of *Artemisia absinthium* contained an enzyme system capable of converting naphthalene acetaldehyde to naphthaleneacetic acid.

Material and methods

Preparations of tryptophol and 1-naphthalene acetaldehyde were obtained through the courtesy of Dr. K. A. Jensen, Department of Chemistry, University of Copenhagen. The crystalline sample of naphthalene acetaldehyde had been prepared according to method no. 3 of JENSEN and CHRISTENSEN (**7**, p. 704; cf. also **8**). Since only milligram-amounts of the aldehyde were available, biological tests were used for its determination. A few milligrams of the aldehyde were dissolved in peroxide-free ether and the solution shaken out with a sodium bicarbonate solution in order to remove possible traces of acid substances. After suitable dilution various amounts of the aldehyde were transferred to agar squares (weighing 100 mg. each) by Boysen Jensen's ether-dropping method (**2**, **3**) and the agar tested on double-decapitated, soil-grown Avena plants. The activity curve for 1-naphthalene acetaldehyde shown in figure 1 is based on the mean results of 50 such tests. A similar curve for 1-naphthaleneacetic acid, based on 32 tests, is shown in the same figure. The curves show that the acid is about 16 times as active as the aldehyde in the Avena test. The Avena curvatures given by the various fractions obtained in the conversion experiments reported below were converted to absolute units (γ) of growth substance by means of these standard curves.

Aqueous solutions of naphthalene acetaldehyde needed for the conversion experiments were obtained by evaporating the ether and extracting the residue with two 5-ml. portions of a freshly prepared 0.01 *M* KH_2PO_4 solution.

Coleoptile juice was prepared from etiolated Avena seedlings cultivated

as ordinary test plants but one day older. The apical part of the coleoptile, 25 mm. long, was cut off and the leaf pulled out. In general, 65 such coleoptiles, weighing on an average 1.2 gm. (fresh weight), were used. They were crushed in a mortar, and 1.2 ml. of a 0.01 *M* KH_2PO_4 solution were added. The resulting brei was filtered through a coarse Pyrex fritted glass filter. The amount of filtrate (in general 1.2 ml.) was measured and twice its volume of the above phosphate buffer was added. The diluted filtrate thus contains roughly 15% coleoptile juice.

In the conversion experiments 1-ml. portions of the diluted coleoptile juice were mixed with 1 ml. of the buffer solution in which naphthalene acetaldehyde had been dissolved. The mixtures (pH = 5.8) were incubated in open test tubes at 22° C in the dark. Control experiments were made with aldehyde mixed with buffer solution instead of coleoptile juice. After various periods of time the mixtures were partitioned between ether and water as follows. The reaction mixture was diluted with 3 ml. of water. One half ml. of a saturated (0.95 *M*) solution of sodium bicarbonate was added. The alkaline solution (pH = 9.4) was shaken with three successive 9-ml. portions of peroxide-free ether. The ether, containing the unconverted aldehyde, was made up to 25 ml. The alkaline, aqueous phase was acidified (to pH = 2.2) with 0.6 ml. 1 *N* HCl and again shaken with three successive 9-ml. portions of ether which was then made up to 25 ml., constituting the acid fraction. (In control experiments with pure naphthaleneacetic acid 95% was recovered by shaking at a pH-value of 2.2 and 91% at a pH-value of 1.2.) In general three or four aliquots from each ether fraction were tested in the Avena test on different days at concentrations yielding curvatures on the ascending part of the standard curves (fig. 1).

Results

The main results are given in table I which shows that naphthalene acetaldehyde disappears very rapidly when diluted coleoptile juice is added. Simultaneously, a considerable amount of acid growth substance, undoubtedly naphthaleneacetic acid, is formed. If the number of moles of acid formed is divided by the number of moles of aldehyde which have disappeared the figures listed in the last column of table I are obtained. The mean of these figures is 0.48 which means that one mole of acid is formed per two moles of aldehyde which disappear.

A number of control experiments were also made. The auxin content of the coleoptile juice was determined. No neutral growth substance was found; but the juice contained an acid auxin which, when calculated as indoleacetic acid, was present in the amount of 0.011 γ per ml. of undiluted juice, corresponding to 1.7×10^{-4} γ per 25-mm. coleoptile. This figure agrees with values obtained by THIMANN (**13**) and WILDMAN and BONNER (**16**). The natural auxin present in 1 ml. of sixfold diluted juice will simulate the effect of 0.017 γ or 0.090 moles $\times 10^{-9}$ of naphthaleneacetic acid. This amount is negligible as compared with the quantities of growth substance added.

The stability of naphthaleneacetic acid in diluted coleoptile juice was

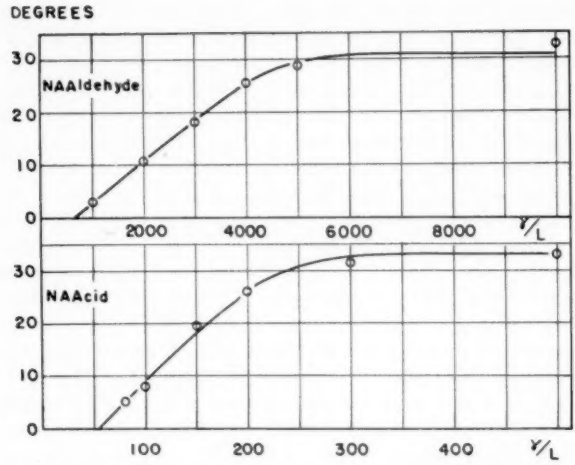


FIG. 1. Relationship between concentration and activity in the Avena test of naphthalene acetaldehyde (above) and naphthaleneacetic acid (below). Ordinate: degrees curvature. Abscissa: concentration of growth substance in the agar blocks.

tested. Samples of 2.31 moles $\times 10^{-9}$ of the acid were mixed with diluted coleoptile juice and treated as in the conversion experiments with aldehyde. No neutral growth substance was formed from the acid. The amounts of acid recovered after 60 and 285 minutes were 2.31 and 2.15 $\times 10^{-9}$ moles, respectively. It may thus be concluded that only negligible amounts of the

TABLE I

CONVERSION OF NAPHTHALENE ACETALDEHYDE TO NAPHTHALENEACETIC ACID IN DILUTED COLEOPTILE JUICE

Initial amount of aldehyde,* M × 10^{-9}	Duration of incubation min.	Aldehyde disappeared, M × 10^{-9}	Acid formed, M × 10^{-9}	Ratio M. acid formed / M. ald. disapp.
10.8	255	9.1	5.2	0.57
14.6	15	13.4	5.1	0.38
14.6	60	12.9	5.7	0.44
15.2	15	12.9	5.2	0.40
15.2	60	14.0	6.6	0.47
25.8	120	20.9	9.5	0.45
25.8	180	22.7	10.5	0.46
73.5	60	41.7	23.1	0.55
103	120	96.2	52.3	0.54
103	255	96.4	49.1	0.51
139	60	65.4	33.5	0.51
139	120	107	56.6	0.53
			Mean:	0.48

*One mole × 10^{-9} = 0.17 γ.

TABLE II

STABILITY OF NAPHTHALENE ACETALDEHYDE IN BUFFER SOLUTION IN ABSENCE OF COLEOPTILE JUICE

Amounts of aldehyde and acid recovered, M × 10^{-9}							
0 min.		60 min.		240 min.		300 min.	
Aldehyde	Acid	Aldehyde	Acid	Aldehyde	Acid	Aldehyde	Acid
10.2	0	9.9	0	10.5	0		
10.4	0			8.8	0.03		
10.4	0.06			10.4	0.20		
27.3	0.13			28.2	0.13	25.4	0.20

acid, if any, are destroyed by the juice within the duration of the experiments. Since indoleacetic acid has been shown to be stable in coleoptile juice (**11**) it is not surprising that naphthaleneacetic acid is also stable. The stability of naphthalene acetaldehyde in buffer solution in the absence of coleoptile juice was tested using the same methods as in the conversion experiments. The results (table II) show that the aldehyde is stable for at least 4 hours in the buffer solution.

If the conversion of naphthalene acetaldehyde to acid is enzymatic, no conversion should take place in boiled coleoptile juice. Table III shows the results of experiments with diluted coleoptile juice which had been boiled for 20 minutes. Practically no acid was formed; but it was impossible to recover all of the added aldehyde when present at low concentrations. At the highest concentration of aldehyde tested no loss occurred.

If a mixture of aldehyde solution and fresh coleoptile juice is shaken out with ether immediately after mixing, it might be possible to recover all of the aldehyde, unconverted. Attempts to recover the added aldehyde at zero time, however, yielded results (table III) very similar to those obtained with boiled coleoptile juice. As expected, practically no acid was found at zero time; but it was impossible to obtain a 100% recovery of the aldehyde at low concentrations. The probable causes of the loss will be discussed later. There is no loss of acid.

TABLE III

THE INFLUENCE OF BOILED AND FRESH COLEOPTILE JUICE ON THE RECOVERY OF NAPHTHALENE ACETALDEHYDE

Initial amount of aldehyde, M × 10^{-9}	Boiled coleoptile juice			Fresh coleoptile juice. Zero time	
	Duration of incubation, min.	M × 10^{-9}		M × 10^{-9}	
		Aldehyde disappeared	Acid formed	Aldehyde disappeared	Acid formed
10.8	155	5.6	0	3.2	0
15.2	107	6.8	0.02	6.3	0.27
25.8	120	3.8	0	4.6	0
139	60	(−1.8)	0	(−8.1)	0.07

The ratio of one mole of acid formed per two moles of aldehyde suggests that the conversion process may be a dismutation in which equivalent amounts of alcohol and acid are formed. The possible interference with the Avena test of the alcohol thus formed was studied with indole-ethyl alcohol (tryptophol) because naphthalene-ethyl alcohol was not available. Tryptophol, which has been shown previously to have a very slight activity in the Avena test (**10**) and the pea test (**14**), was required at a concentration of 200,000 γ/l. to give a curvature of 5° in the Avena test. Hence, the activity of tryptophol is only 0.005% of the activity of indoleacetic acid, or 0.05% that of indole acetaldehyde. The maximum curvature which tryptophol can produce is 8.5°, and 450,000 γ/l. are required to give this angle. Since the activities of naphthalene compounds in the Avena test have always been found to be lower than those of the corresponding indole compounds, there is good reason to believe that naphthalene-ethyl alcohol, if active at all, will show a still lower activity than tryptophol in the Avena test. The growth-promoting activity of the quantities of alcohol possibly formed in the conversion experiments, therefore, is negligible as compared with the amounts of acid and aldehyde involved. Since the neutral and acid growth substances were separated before bio-assay, any alcohol present would interfere only with the determination of the aldehyde, not the acid. The possibility that the alcohol formed might have an inhibitory effect in the Avena test was tested with mixtures of tryptophol and indole acetaldehyde. The concentration of the latter was determined by bio-assay, using the factor of 9 (see later) for a conversion of γ-"equivalents" to actual γ. It was found that the admixture of 9100 or 91000 γ/l. of tryptophol to 140 γ/l. of indole acetaldehyde did not change the curvature of 12° given by 140 γ/l. of indole acetaldehyde alone. The experiments with tryptophol give no indication that naphthalene-ethyl alcohol, if formed in the conversion experiments, would interfere with the determination of naphthalene acetaldehyde.

Discussion and conclusions

Upon addition of diluted coleoptile juice to a solution of naphthalene acetaldehyde the aldehyde starts to disappear. Within a few minutes, the formation of an acid growth substance, undoubtedly naphthaleneacetic acid, can be demonstrated. The formation of the acid is evidently an enzymatic process since no acid is formed when boiled juice is added to the aldehyde solution.

The amount of aldehyde initially present per test tube in the conversion experiments was determined by bio-assay of a sample of the aldehyde solution to which no coleoptile juice had been added. The amount of aldehyde which had disappeared was computed as the amount initially present minus the amount recovered. The data presented in table I show that at any moment, after 15 minutes have elapsed, there is present in the solution an amount of naphthaleneacetic acid which is very close to one half of the amount of aldehyde that has disappeared. The question now is whether all

of the aldehyde which has disappeared was actually functioning as a substrate for the conversion enzyme. In attempts to recover the aldehyde immediately after addition of fresh coleoptile juice, it was found that part of the aldehyde had disappeared (either instantaneously or during the first shaking with ether, which lasts four minutes) without giving rise to the formation of acid growth substance. The aldehyde showed a similar behavior during a prolonged treatment with boiled coleoptile juice. Various explanations for the loss of recoverable aldehyde may be offered. The loss may in both cases be the result of an adsorption of aldehyde to protein in the juice or of a specific reaction between aldehyde and protein or other constituents of the juice leading to fixation of aldehyde. A highly probable explanation in the case of fresh coleoptile juice is that the aldehyde combines with the enzyme faster than the acid is released and that it is impossible to remove the aldehyde from the enzyme-substrate complex with ether. It was shown by STERN (**12**) that catalase and monoethyl hydrogen peroxide form an enzyme-substrate complex within a few seconds after mixing, while the enzymatic breakdown of this complex requires minutes. CHANCE (**4**) studied the kinetics of the formation and enzymatic breakdown of the peroxidase-hydrogen-peroxide complex and found (**4**, fig. 6) that the formation of the enzyme-substrate complex took place in a fraction of a second, whereas the breakdown required several seconds, the actual time depending on the concentration of the oxygen acceptor (ascorbic acid). When the concentration of ascorbic acid was $2.9\ M \times 10^{-6}$, slightly lower than that of the substrate, 50% of the enzyme-substrate complex was still present after ten seconds. If the enzymatic formation of naphthaleneacetic acid from the aldehyde is a dismutation, the aldehyde itself functioning as an acceptor (here hydrogen acceptor), one would actually expect a relatively long life of the (hypothetical) enzyme-substrate complex in the experiments reported here, because the concentration of acceptor was low.

The loss of aldehyde in the experiments with boiled coleoptile juice is perhaps not comparable with that occurring when fresh juice was used, because the aldehyde was incubated with the boiled juice for a prolonged period of time. The possibility exists, however, that the denaturated enzyme is capable of forming a complex with the aldehyde, but not to convert it into alcohol and acid.

Figure 2 shows the effect of computing the amount of aldehyde disappearing on the basis of the amount recoverable at zero time. On this basis the molar yield would be decreasing with increasing amounts of aldehyde converted. Such a decrease seems rather unlikely and, together with the above considerations, makes the assumption that all of the added aldehyde was available to the conversion enzyme appear the more probable one.

For further studies of the mechanism of the conversion, the use of partially purified enzyme preparations will probably be advantageous. Such preparations might also be used for routine conversion of indole acetaldehyde to indoleacetic acid before bio-assay of impure plant extracts, a pro-

cedure suggested by Dr. S. A. Gordon, who found that lyophilized coleoptile juice and fractions precipitated by alcohol, acetone or ammonium sulphate contained the active enzyme system (unpublished results).

Even though the results still leave some doubt in regard to the mechanism of the enzymatic conversion they are adequate for the principal purpose of the present study, *viz.*, to determine the weight of one γ-"equivalent" of indole acetaldehyde. The calculation of this value requires only determinations of the actual yields of acid per unit of aldehyde disappearing during the experiment, and these yields are quite constant for a given aldehyde (table I and fig. 2). Except for the use of a more dilute coleoptile juice, the

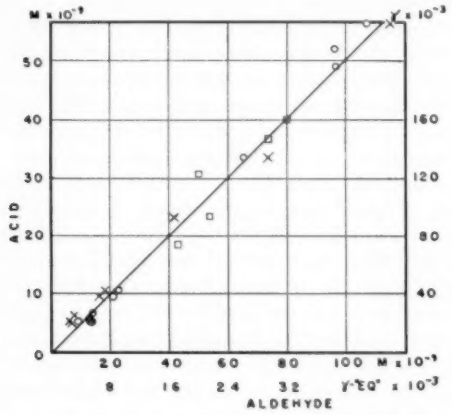


FIG. 2. *Circles* and *crosses* (left-hand ordinate, upper abscissa): yield of naphthaleneacetic acid plotted against amount of naphthalene acetaldehyde disappeared. Aldehyde values based either on amount actually added (circles) or on amounts recovered at zero time (crosses). The line represents the theoretical yield of 1 mole of acid per 2 moles of aldehyde.

Squares (right-hand ordinate, lower abscissa): yield of indoleacetic acid plotted against γ-"equivalents" of indole acetaldehyde disappeared [data from (**11**)]. The line represents a yield of 5 γ of acid per γ-"equivalent" of aldehyde.

experiments with naphthalene acetaldehyde were carried out in the same manner as the experiments with indole acetaldehyde previously reported (**11**), which showed that one γ-"equivalent" of indole acetaldehyde yielded on an average 4.9 γ of indoleacetic acid by identical treatment (compare fig. 2). Again the yield is based upon bio-assay of the amount of aldehyde added, not on the amount recoverable at the so-called zero time. The amount of 4.9 γ is equivalent to 28 moles $\times 10^{-9}$ of indoleacetic acid. Making the reasonable assumption that 2 moles of either of the two aldehydes yield one mole of acid under the influence of the enzyme system in coleoptile juice, it may be concluded that the 28 moles $\times 10^{-9}$ of indoleacetic acid origi-

nated from 56×10^{-9} moles or 8.9 γ of indole acetaldehyde. One γ-"equivalent" of indole acetaldehyde, therefore, has a weight of approximately 9 γ; or in other words, on the above assumption approximately 9 γ of indole acetaldehyde are required to give the same effect in the Avena test as 1 γ of indoleacetic acid.

Indole acetaldehyde thus has an activity in the Avena test of 11% of that of indoleacetic acid. A comparison of the relative activities of two other acid growth substances and their corresponding aldehydes is made in table IV. A similar comparison, made previously (**10**), was based on a somewhat less sensitive modification of the Avena test. The concentrations required for a 5° curvature, as listed in table IV, therefore, are somewhat lower than the ones previously reported. In the case of naphthalene acetaldehyde, however, the main reason for the low activity reported in the previous study was the presence of impurities in the preparation.

TABLE IV

ACTIVITIES OF THREE ACID GROWTH SUBSTANCES AND THEIR CORRESPONDING ALDEHYDES

	Avena test		Artemisia root test (1)			
	Concentration required for 5° curvature, γ/L.	Relative activity (acid = 100)	Concentration required for 50% inhibition, γ/L.		Relative activity (acid = 100)	
			4 hrs.	24 hrs.	4 hrs.	24 hrs.
3-Indoleacetic acid	10.5	100	35.5	14.1	100	100
3-Indole acetaldehyde	95*	11.0†	114	186	30.6	7.6
1-Naphthaleneacetic acid	80	100	20.0	159	100	100
1-Naphthalene acetaldehyde	1250	6.4	2240	3160	0.9	5.0
Phenylacetic acid	1.9×10^6	100				
Phenyl acetaldehyde	26×10^6	7.3				

*3-Indole-ethyl alcohol: 200,000.
†3-Indole-ethyl alcohol: 0.005.

If the activity of each of the acids is set at 100, the activities of indole-, naphthalene-, and phenyl-acetaldehyde are 11, 6, and 7, respectively. Although not identical, these three figures are of the same order of magnitude. The activities of indoleacetic and naphthaleneacetic acids and their corresponding aldehydes in inhibiting the elongation of Artemisia roots were determined by ASHBY (**1**). His results, computed on the same basis as the results obtained in the Avena test, are given in table IV for comparison. In the Artemisia test the relative activities, measured after 24 hours, proved to be of the same order of magnitude as in the Avena test. When measured after four hours, on the other hand, the relative activities of the two aldehydes are quite different. VELDSTRA and BOOIJ (**14**) found naphthalene

acetaldehyde to be nearly as active as naphthaleneacetic acid in the pea test.

In view of the 50% yield of acid in the conversion experiments, the question arises as to why the concentration of aldehyde in the agar blocks has to be 9 to 16 times as high as that of the acids in order to produce the same curvature in the Avena test. It must be assumed that the rate of conversion of the aldehydes increases with their concentration. Part of the answer to the above question, therefore, is probably that only at concentrations several times those required for the acids will the rate of conversion become sufficient to build up the necessary amount of the active acid. In addition, differences in transportability of aldehyde and acid may play an important role.

Summary

1. Curves showing the relationship between concentration and activity in the Avena test of 1-naphthalene acetaldehyde and 1-naphthaleneacetic acid were constructed.

2. An enzyme system present in fresh coleoptile juice is capable of converting naphthalene acetaldehyde to naphthaleneacetic acid yielding 1 mole of acid per 2 moles of aldehyde.

3. Using the same methods as in the present study it was shown previously (**11**) that the arbitrary unit of one γ-"equivalent" of indole acetaldehyde yielded 28×10^{-9} moles ($= 4.9\,\gamma$) of indoleacetic acid when treated with coleoptile juice. On the basis of the molar yield obtained in the present study of the naphthalene analogue it was concluded that the 28×10^{-9} moles of acid originated from 56×10^{-9} moles of indole acetaldehyde. One γ-"equivalent" of indole acetaldehyde, therefore, would be equal to 56×10^{-9} moles or approximately $9\,\gamma$.

4. A comparison of the activities of indole-, naphthalene-, and phenylacetic acids and their corresponding aldehydes is made. The aldehydes show an activity in the Avena test which is 6 to 11% of that of the acids.

The present work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Department of Botany
University of Chicago
Chicago, Illinois

LITERATURE CITED

1. Ashby, W. C. Effects of certain acid growth-regulating substances and their corresponding aldehydes on the growth of roots. Bot. Gaz. **112**: 237–250. 1951.
2. Boysen Jensen, P. Über eine Mikromethode zur quantitativen Bestimmung der Wuchsstoffe der A-Gruppe. Planta **26**: 584–594. 1937.

3. Boysen Jensen, P. Quantitative Bestimmung der beschleunigenden Streckungs-Wuchsstoffe in der sauren Fraktion der Ätherextrakte aus höheren Pflanzen. Planta **31**: 653–669. 1941.
4. Chance, B. The kinetics of the enzyme-substrate compound of peroxidase. Jour. Biol. Chem. **151**: 553–577. 1943.
5. Gordon, S. A. and Nieva, F. S. The biosynthesis of auxin in the vegetative pineapple. I. Nature of the active auxin. Arch. Biochem. **20**: 356–366. 1949.
6. Gordon, S. A. and Nieva, F. S. The biosynthesis of auxin in the vegetative pineapple. II. The precursors of indoleacetic acid. Arch. Biochem. **20**: 367–385. 1949.
7. Jensen, K. A. and Christensen, S. A. K. Researches on plant growth substances. II. On 1-naphthylacetaldehyde 2. Acta Chem. Scandinavica **4**: 703–709. 1950.
8. Jensen, K. A. and Dynesen, E. Researches on plant growth substances. I. On 1-naphthylacetaldehyde 1. Acta Chem. Scandinavica **4**: 692–702. 1950.
9. Larsen, P. 3-Indole acetaldehyde as a growth hormone in higher plants. Dansk Bot. Arkiv **11**(9): 1–132. 1944.
10. Larsen, P. Neutral auxins. Nature **159**: 842. 1947.
11. Larsen, P. Conversion of indole acetaldehyde to indoleacetic acid in excised coleoptiles and in coleoptile juice. Amer. Jour. Bot. **36**: 32–41. 1949.
12. Stern, K. G. On the mechanism of enzyme action. A study of the decomposition of monoethyl hydrogen peroxide by catalase and of an intermediate enzyme-substrate compound. Jour. Biol. Chem. **114**: 473–494. 1936.
13. Thimann, K. V. Studies on the growth hormone of plants. VI. The distribution of the growth substance in plant tissues. Jour. Gen. Physiol. **18**: 23–34. 1934.
14. Veldstra, H. and Booij, H. L. Researches on plant growth regulators. XVII. Structure and activity. On the mechanism of the action III. Biochim. et Biophys. Acta **3**: 278–312. 1949.
15. Warmke, H. E. and Warmke, Germaine L. The role of auxin in the differentiation of root and shoot primordia from root cuttings of Taraxacum and Cichorium. Amer. Jour. Bot. **37**: 272–280. 1950.
16. Wildman, S. G. and Bonner, J. Observations on the chemical nature and formation of auxin in the Avena coleoptile. Amer. Jour. Bot. **35**: 740–746. 1948.
17. Wildman, S. G., Ferri, M. G., and Bonner, J. The enzymatic conversion of tryptophan to auxin by spinach leaves. Arch. Biochem. **13**: 131–144. 1947.

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES ACCOMPANYING POLLINATION IN ORCHID FLOWERS. II. RESPIRATION, CATALASE ACTIVITY, AND CHEMICAL CONSTITUENTS

TSUNG-HSUN T. HSIANG[1]

(WITH TWO FIGURES)

Received September 11, 1950

In a previous paper of this series (**19**), data have been presented to show that pollination in orchid flowers brings about considerable changes in their water balance. The present paper deals with the change in respiratory activity, catalase activity, and chemical constituents as consequences of pollination and auxin treatment. The general methods used in sampling and treatment of flowers are essentially the same as that earlier described (**19**). A general conclusion, based on all experimental facts presented in both papers, is also given here.

Respiration

WHITE (**35**) noted an increase of respiratory rate and also of respiratory quotient after pollination in a number of plant species. A critical examination of her data, however, would seem to show that the respiratory quotient was unusually low for the non-pollinated plants, indicating that some constant errors might have been involved. Moreover, in most of the plants she used, fertilization is known to follow pollination more or less closely. It is therefore not safe to attribute the observed effects, if valid, to pollination alone. Further work is needed on the effect of pollination on respiration.

In the work reported here respiration was measured as the amount of O_2 taken up per unit of time per unit of dry weight. A Warburg respirometer of the constant volume type was employed. All experiments were run at $25° \pm 0.2°$ C.

Preliminary study showed that when tissue sections were floated on phosphate buffer solution at a pH of 5.8 the liquid tended to enter the intercellular spaces and gave the tissues a translucent appearance. Therefore, the use of the buffer solution was omitted and the tissues were put directly into the flasks without any added liquid. Two tenths of a milliliter of 20% KOH on filter paper was used in the center well. After equilibration, readings were taken at 15- or 30-minute intervals until several constant values had been obtained. An average reading of four to six intervals was usually taken to represent the respiration value.

The respiratory quotient was obtained by using the same sample in the same flask, instead of using two separate flasks. Readings were first taken without KOH in the center well; KOH was then added and readings were

[1] Present address: Department of Biology, Morehouse College, Atlanta, Ga.

taken again after re-equilibration. After the respiration study, the tissues were carefully taken from the flasks and dried at 80 to 100° C for 24 hours to obtain the dry weights.

Displacement experiments showed that the fresh weight of the floral tissue was numerically close to its volume. A sample flower of Cymbidium weighed 2.76 grams and, when immersed in water in a measuring cylinder, the water level in the cylinder rose 2.95 ml. The volume to weight ratio was therefore 1.07. The value is close enough to unity to permit taking the fresh weight of the flower as approximately equivalent to the volume.

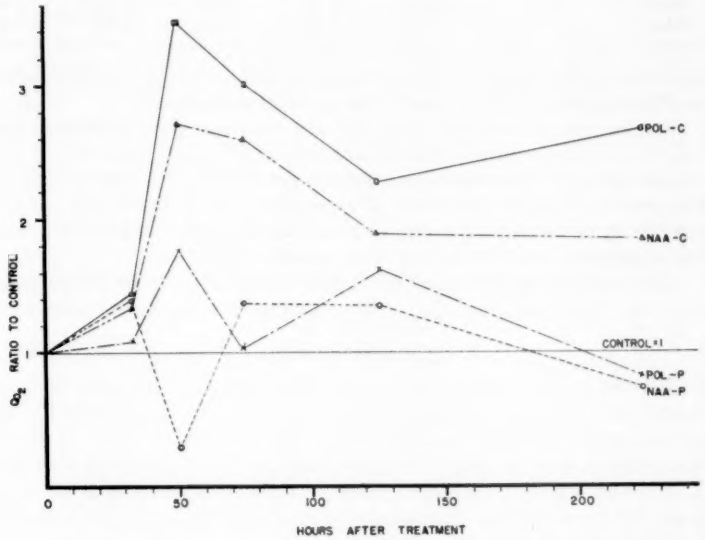


FIG. 1. Change in respiratory rate (mm.^{3} O_2/gm. dry wt./hr.) as ratio to control following pollination or auxin treatment. *Cymbidium Lowianum*.

The readings in mm. pressure were converted into Q_{O_2}, defined as mm.^{3}/mg. dry material/hr., in the manner given by UMBREIT, BURRIS, and STAUFFER (**32**). Results with Cymbidium are shown in table I. When the Q_{O_2} of the control was arbitrarily fixed as 1, the ratios of the other values to this could be obtained easily. Figure 1 was constructed by plotting these ratios against time.

In *Cymbidium Lowianum* (fig. 1), a threefold increase in respiration was shown by the columns 48 hours after pollination. A slight depression was again followed by a gradual rise which lasted to the end of the experiment. The NAA-C curve followed the same trend except that it did not show any sign of rising after the drop. The sudden depression below the control of the NAA-P curve seems rather unexpected. However, experimental results

TABLE I

EFFECT OF POLLINATION AND AUXIN TREATMENT ON RESPIRATORY RATE EXPRESSED AS Q_{O_2} (MM^3/MG. DRY MATERIAL/HR.)

Part and Treatment	Hours after treatment					
	0	32	44.5	73.5	125	222
Cont-P	1.22	1.22	0.81	1.13	0.75	1.03
Pol-P		1.33	1.43	1.16	1.22	0.87
NAA-P		1.45	0.24	1.56	1.02	0.76
Cont-C	0.64	0.74	0.48	0.38	0.50	0.56
Pol-P		1.06	1.67	1.13	1.14	1.48
NAA-C		0.99	1.30	0.98	0.95	1.08

show that a depression following stimulation also occurred with respiration in Phalaenopsis, indicating more than an experimental error.

Studies with *Coelogyne Mooreana* and *Cattleya Bowringiana* all exhibited an approximately twofold increase in the respiration rate of the treated columns. The experiment done on cut flowers of a hybrid Cattleya also showed a slight stimulation of respiration. It is noted that marked stimulation of respiration in the column usually occurred before there was any appreciable increase in fresh weight (table II).

The effects of pollination on the R.Q. of the floral parts are tabulated in table III. Obviously the R.Q. was not significantly influenced by pollination.

Catalase activity

Activity of this enzyme was expressed as milliliters of oxygen evolved from added hydrogen peroxide per unit of fresh weight per minute. A known amount of tissue was ground in a mortar with an excess of $CaCl_2$ until reduced to a fine pulp. This was transferred to a graduated tube and diluted with water to the 10-ml. mark. Duplicate aliquots of 2 ml. were taken for determination. The apparatus used was similar to that described by Loomis and Shull (**21**).

TABLE II

COMPARISON OF PER CENT. INCREASE IN FRESH WEIGHT AND PER CENT. INCREASE IN RESPIRATION AS A CONSEQUENCE OF POLLINATION.
Cattleya Bowringiana.

Part and Treatment	Hours after treatment					
	34		47		99	
	Fr. wt. % cont.	Resp'n. % cont.	Fr. wt. % cont.	Resp'n. % cont.	Fr. wt. % cont.	Resp'n. % cont.
Cont-P	100	100	100	100	100	100
Pol-P	117	147	94.5	132	74.5	50
Cont-C	100	100	100	100	100	100
Pol-C	99.8	144	108	150	112	216

TABLE III

EFFECT OF POLLINATION ON THE RESPIRATORY QUOTIENT (R. Q.) OF THE FLORAL PARTS

Species	Part and Treatment	With KOH		Without KOH			R. Q.
		h (mm.)	mm³ (h × K_{O_2})	h (mm.)	Diff. (mm.)	mm³ CO_2 (Diff. × K_{O_2})	
Coelogyne Mooreana (12.5 hrs. after treatment)	Cont-C	3.3	5.58	0.4	2.9	4.99	0.90
	Pol-C	7.2	11.09	0.5	6.7	10.59	0.95
Cymbidium Lowianum (44 hrs. after treatment)	Cont-P	56.1	85.27	7.0	49.1	81.50	0.96
	Pol-P	60.0	85.80	5.1	54.9	86.74	1.01
	Cont-C	37.5	60.38	5.5	32.0	54.40	0.90
	Pol-C	43.1	62.06	6.0	37.0	57.72	0.93

The change in catalase activity caused by pollination and NAA treatment in *Cymbidium Lowianum* is shown in figure 2. A similar depression after rapid stimulation of catalase activity, as occurred with oxygen uptake is observed. An increase in catalase activity of floral parts was also noted when *Dendrobium nobile* was pollinated with Phalaenopsis pollen.

Total phosphorus

FISKE and SUBBAROW's method (**16**) for total phosphorus determination was followed. Galvanometric readings were taken with a Klett-Summerson type photocolorimeter at 476 mμ 30 minutes after the addition of the reagents.

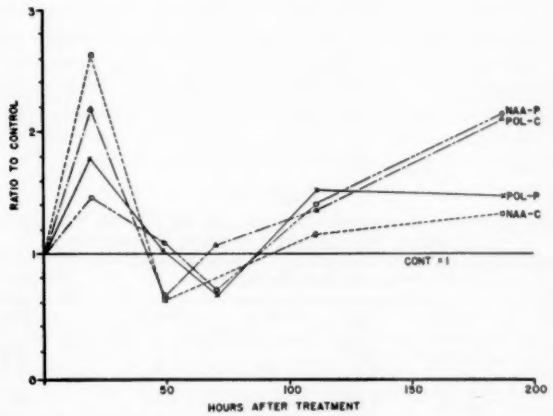


FIG. 2. Change in catalase activity (ml. O_2/gm. fresh wt./min.) as ratio to control following pollination or auxin treatment. *Cymbidium Lowianum*.

TABLE IV

CHANGE OF TOTAL PHOSPHORUS (MG./GM. DRY WT.) IN THE FLORAL PARTS FOLLOWING POLLINATION AND AUXIN TREATMENT. *Cattleya labiata.*

Part and Treatment	Hours after treatment				Dry wt. at end of 139 hrs. (gm.)
	0	31	139	167	
Cont-C	2.0	2.2	1.4	1.8	0.1994
Pol-C		2.6	2.6	3.4	0.3188
NAA-C		2.8	2.8	3.0	0.2990
IAA-C		2.5	2.5	0.7	0.2009
Cont-P	2.3	2.3	2.3	2.5	0.4167
Pol-P		1.4	0.27	0.45	0.2785
NAA-P		1.7	0.25	0.52	0.2929
IAA-P		1.8	0.30	0.38	0.2073

Three methods were used for ashing the plant material. They were: dry ashing by fusion in an electric muffle at 600° C; wet ashing by concentrated HNO_3 and perchloric acid; and by concentrated H_2SO_4 and 30% H_2O_2 (**36**). Comparison of results of the three ashing methods shows good agreement.

Tables IV and V show respectively the change in total phosphorus content in the floral parts of *Cattleya labiata* and *C. Bowringiana* following various treatments.

Comparison of the behavior of flowers of both species of Cattleya reveals the following facts: (1) While in *Cattleya labiata* total phosphorus is distributed evenly between the column and the perianth, it is more or less concentrated in the column in *C. Bowringiana;* (2) the total phosphorus content of the treated perianth in both species underwent a sharp drop as the total phosphorus content of the corresponding columns was increasing; (3) in *C. labiata*, the actual loss of phosphorus in the perianth was greater than the gain in the column, resulting in a net loss per flower; (4) in both species, the pollinated columns had approximately a 200% increase in total phosphorus on unit dry weight basis, and a 300% increase on a per flower basis.

TABLE V

CHANGE OF TOTAL PHOSPHORUS (MG./GM. DRY WT.) IN THE FLORAL PARTS FOLLOWING POLLINATION. *Cattleya Bowringiana.*

Part and Treatment	Hours after treatment				Total dry wt. (gm.) of 5 flowers at end of 139.5 hrs.
	43	71	95.5	139.5	
Cont-C	3.1	3.1	....	2.7	0.2086
Pol-C	3.5	3.7	4.7	5.2	0.3607
Cont-P	1.28	1.17	1.25	1.08	0.2378
Pol-P	0.58	0.66	0.46	0.50	0.2169

TABLE VI

EFFECT OF POLLINATION AND AUXIN TREATMENT ON TOTAL NITROGEN CONTENT OF PERIANTH. *Cattleya labiata.* 167 HOURS AFTER TREATMENT.

Part and Treatment	Total N % dry wt.	Total N % cont.	Dry wt. % cont.	Dry wt. at end of expt. (gm.)	mg. N per flower
Cont-P	1.56	100	100	0.4685	7.5
Pol-P	0.76	47.36	62.62	0.2934	2.2
NAA-P	0.88	54.83	44.89	0.2103	1.8

Total nitrogen

Total nitrogen of the floral parts was determined by Friedrich's micro-Kjeldahl method given by PREGL (**28**). Twenty milligrams of the oven-dried, finely ground material were used for each determination. The results are summarized in tables VI and VII.

It is clear that pollination induces mobilization of nitrogen from the perianth to the column. For comparison, the relative increase and decrease of total dry weights corresponding to the nitrogen changes are also shown in the tables. The data suggest that the nitrogen gain of the column was preferentially affected by pollination, but not by NAA treatment.

Sugars

HASSID'S (**18**) ceric sulphate method of sugar determination was adapted to a semi-micro level. One tenth of a gram of the dried material was extracted with 80% methyl alcohol for six hours in a Soxhlet extractor. The extract was evaporated to a volume of about 10 ml., clarified, decolorized and filtered in the usual manner. The filtrates, together with the washings, were made to 100 ml. Aliquots of 10 ml. equivalent to 10 mg. of dry material were taken for analysis.

For sucrose, the acid hydrolysis method of LEHMANN (**20**) was found satisfactory. Fifty milliliters of the extract was incubated with 2 ml. of concentrated HCl at 70° C for 15 minutes. After cooling, the contents were neutralized with 15% NaOH and made up to 100 ml. Aliquots equivalent to 5 mg. of dry material were taken for reducing sugar determination as before.

TABLE VII

EFFECT OF POLLINATION AND AUXIN TREATMENT ON TOTAL NITROGEN CONTENT OF COLUMN. *Cattleya labiata.* 91 HOURS AFTER TREATMENT.

Part and Treatment	Total N % dry wt.	Total N % cont.	Dry wt. % cont.	Dry wt. at end of expt. (gm.)	mg. N per flower
Cont-C	1.43	100	100	0.1368	1.96
Pol-C	3.55	241.2	161	0.2203	7.82
NAA-C	2.19	153.1	177	0.2432	5.33

TABLE VIII

EFFECT OF POLLINATION ON SUGAR CONTENT (MG./GM. DRY WT.) OF FLORAL PARTS. *Cattleya Bowringiana.*

Part and Treatment	Initial			At end of 137.5 hrs.		
	Reducing	Sucrose	Total	Reducing	Sucrose	Total
Cont-P	76	14	90	84	14	98
Pol-P				78	25	103
Cont-C	109	20	129	146	7	153
Pol-C				146	30	176
mg. sugar in 6 cont. flowers	44.46	8.17	52.63	52.07	5.43	57.50
mg. sugar in 6 pol. flowers				53.44	13.61	67.05

The results obtained are tabulated in tables VIII and IX. In both species studied, the perianth did not undergo any appreciable change in either its reducing or non-reducing sugar content. In *Cattleya Bowringiana*, pollination did not affect the reducing sugar concentration, but increased the sucrose about fourfold. On the other hand, in *Cymbidium Lowianum*, pollination brought about a considerable increase in reducing sugar and a decrease in sucrose. The experimental data are also calculated on the basis of one flower and a group of flowers.

Starch

SESHAGIRIAH (**29**) observed an abundance of starch in the ovary sections of pollinated flowers of *Habenaria longicalcarata*, in contrast to its entire

TABLE IX

EFFECT OF POLLINATION AND AUXIN TREATMENT ON SUGAR CONTENT (MG./GM. DRY WT.) OF FLORAL PARTS. *Cymbidium Lowianum.* 73.5 AND 222 HOURS AFTER TREATMENT.

Part and Treatment	73.5 hours			222 hours		
	Reducing	Sucrose	Total	Reducing	Sucrose	Total
Cont-P				48.58	17.48	66.06
Pol-P				49.69	9.77	59.46
NAA-P				41.88	15.73	57.61
Cont-C	24.28	30.20	54.48	46.87	20.33	67.20
Pol-C	28.82	11.28	40.10	68.89	11.75	80.64
NAA-C	26.66	21.99	48.65	....	....	....
mg. sugar in whole cont. flower				0.94	0.37	1.31
mg. sugar in whole pol. flower				1.83	0.33	2.16

absence in those of non-pollinated flowers. He concluded that the substances from the pollen stimulated starch synthesis in the ovary.

In the present investigation the same observation was also made. Ovaries were sectioned and examined for starch by the usual staining technique with KI-I solution. It was found that flowers which had just opened always had abundant starch in a special layer of cells running almost continuously in the ovary wall outside the vascular tissues. As the flower ages, the starch grains gradually diminish and then disappear completely. However, when the flowers were pollinated or treated with auxin, the starch grains remained present. This was observed in *Dendrobium thrysifolium, D. nobile* and *Cymbidium Lowianum*.

It was first thought that the state of anabolism induced by pollen or auxin treatment might make available an external supply of foodstuffs, presumably from the vegetative parts, thus sparing the reserve starch. However, observations made on cut flowers did not support this hypothesis. Flowers that were pollinated after they were cut from the plant and put in water were still rich in starch 115 hours after pollination, at which time the controls had lost starch.

Discussion

WHITE'S (**33**) results on respiration of pollinated flowers were partly confirmed in the present work. An increased oxygen uptake following pollination was invariably noticed. This pollen-stimulated respiration was duplicated by application of auxin. As to the known effect of auxin on respiration, a definite relationship has not been established. Much has been done on Avena coleoptiles, with apparently more or less conflicting results (**4, 5, 6, 7, 8, 14**). In plants other than Avena, BROWN (**9**), SMITH, HAMNER, and CARLSON (**30**) have reported an acceleration of respiration by 2,4-D and a consequent depletion of food reserve. MITCHELL, BURRIS, and RIKER (**23**) observed a definite inhibitory effect of various growth regulators on the respiration of tomato stem slices and other stem and root tissues.

The rise of respiratory quotient following pollination, as reported by WHITE (**35**), was not found by the present author. Neither pollination nor auxin treatment significantly altered the R.Q. of the floral parts. A value in the neighborhood of one was obtained, indicating the dominance of carbohydrate metabolism. It was shown that the stimulation of respiration normally occurred before there was any appreciable increase in fresh weight which, in turn, usually preceded the dry weight increase. This seems to imply that increased respiration is necessary for the accelerated water uptake, which is in line with the fact revealed in the water relation studies (**19**), that part of the augmented water uptake is dependent upon aerobic respiration.

The observation that both oxygen uptake and catalase activity fell below normal after rapid stimulation, as shown in figures 1 and 2, seems to indicate the operation of some limiting factor or factors. The factors might represent substances used up during the stimulation phase. A detailed re-

view of the literature dealing with the correlation of catalase activity with respiratory activity is found in MILLER's book (**22**). It is reported that most investigators found a positive correlation between them, although negative correlation and total absence of correlation have also been reported. The results presented in the present paper demonstrate that the rise in catalase activity precedes the stimulation of respiration. In *Cymbidium Lowianum*, the activity of catalase reached its maximum about 20 hours after treatment, at which time the rate of oxygen uptake was just beginning to rise; when the latter came to its peak, the former fell below normal.

Many papers have appeared dealing with the change in chemical composition of plants following auxin treatment. Application of IAA to stems of bean seedlings causes a mobilization of sugars, chiefly glucose, and soluble nitrogenous compounds to the treated region from other parts of the plants (**31, 24**). More recently, BRUNSTETTER and coworkers (**10**) have studied the change of nine different kinds of minerals in bean stems treated with IAA. Significant increases in all but Ca were observed. They were in order of decreasing response: K, Mg, Mn, B, P, Cu, Fe, and Al.

The results of the present investigation confirmed these findings. Application of auxin or pollen to the stigma of the orchid flower is shown to cause the mobilization of sugar from the vegetative parts and of nitrogen and phosphorus from perhaps both the perianth and the vegetative parts to the column. That the nitrogenous compounds are able to redistribute themselves within the floral parts was early demonstrated by COMBES (**11**). The net loss of total phosphorus from the flower of *Cattleya labiata* after pollination may be compared with the fact cited by BAKUYZEN (**1**) that in wheat nutrient elements were lost from the tops to the roots after flowering. The qualitative difference of the two species of orchids, Cattleya and Cymbidium, in the change of their sugar fractions may be of particular significance when one considers its possible bearing on the appearance of anthocyanin in the lip and the column of the Cymbidium flower.

Relative to the presence of starch in the ovary of the pollinated flower, unless Habenaria is different from Cymbidium and Dendrobium, SESHAGIRIAH's (**29**) observation seems to be incomplete. The slow disappearance of starch in the auxin-treated ovary cannot be reconciled with the results of MITCHELL and WHITEHEAD (**25**), BEAL (**3**) and BAUSOR (**2**). These workers reported an accelerated starch hydrolysis in auxin-treated plants. A possible explanation is that auxin inhibited the activity of diastase. EYSTER (**15**) reported that auxin increased the release of diastase from a bound to an active form, but retarded the activity of the free enzyme. Assuming this enzyme is present in the orchid ovary mostly in free form, the above observations can then be easily explained.

The data presented in this paper and the previous paper (**19**) show that the transfer of pollen to the stigma brings about marked changes in the physiology of the flower within several hours. It is the present intention, by correlating these changes with one another, to form a more continuous

and complete picture and to consider the problem of pollination from a theoretical aspect.

The earliest change detected in the column of pollinated flowers seems to be the increase in catalase activity, closely followed by a stimulation of respiration. The stimulation of respiration is usually apparent before there is any appreciable increase in fresh weight of the tissues. It is therefore logical to conclude that respiration furnishes the energy for the accelerated water uptake. The aerobic nature of the pollen-stimulated water uptake is cletarly demonstrated in the experiment with cut discs of columns of pollinated flowers (**19**). In air such discs absorb more water than those from columns of non-pollinated flowers. In nitrogen they fail to absorb any more water than the discs from columns of non-pollinated flowers.

On a percentage basis, pollination at first brings about an increase in water uptake without an equally great increase in dry matter uptake. This results in an increased succulence of the column tissue. This relationship, however, does not persist long, because the per cent. increase in solid matter soon overtakes the per cent. increase in water, with the consequence that the tissue has a higher percentage of dry matter. This increase in salt uptake and perhaps also the hydrolysis of some of the pre-existing compounds thus increases the osmotic value of the column tissue. Osmotic pressure measured 115 hours after treatment show a slight increase over the control.

Linking together the chain of reactions in the column, we have, in the order of their detected appearance: increase in catalase activity; stimulation of respiration; acceleration of water uptake; increase of uptake of inorganic salts and mobilization of sugars; increase in hydrophilic colloids (speculative); increase in osmotic pressure. The perianth enters into a state of senescence immediately after pollination: it wilts because of water loss through transpiration; its respiration is stimulated for a short time, then undergoes a permanent drop; part of its nutrient content is then transported to the column.

By what mechanism does the pollen act on the flowers? There is no doubt that the orchid pollen is a rich source of auxin. In fact, it has been an important material in the development of auxin concepts. The question is whether the mere diffusion of free auxin from the pollen is sufficient to account for all the observed changes.

From his study of parthenocarpy, GUSTAFSON (**17**) has postulated the theory that pollen normally furnishes growth substances for the initial development of the fruit, the developing ovules and embryos then form new centers for the production of hormones which are responsible for the continued growth of the ovary. This theory, however, does not apply very well in the case of the orchids, since the "new center" will not be formed until a long time after pollination.

VAN OVERBEEK *et al.* (**34**) stated that the amount of auxin in the pollen in normal pollination in Datura was not enough to be the sole source of fruit development, and concluded that the pollen might carry prosthetic

groups for enzymatic activation of auxin precursors in the ovary. MUIR (**26**) observed in tobacco a gradual increase of auxin concentration along the path that the pollen tubes travel. More recently (**27**), he further demonstrated a considerable increase of auxin when pollen extract and ovary extract were brought together, each of which alone produces a much smaller curvature in the Avena test plants.

In the present study, auxin, at the particular concentration used, duplicates or even exceeds the immediate pollination effect in producing the external visible changes. As to the other changes, it was invariably shown that 0.5% NAA in lanolin has a greater effect than pollen in the initial phase of each reaction. Nevertheless, the effect of auxin always ends in a leveling off. On the other hand, the pollen reaction always starts out slowly, but becomes stronger later on.

The limitation of the auxin reaction can be easily explained by the exhaustion of the substance; but to interpret the progressive increase of the pollen reaction, one is forced to assume that it is not only the free auxin contained in the pollen that is responsible for the whole series of changes, but there must be new formation or release of available auxin as the process proceeds. Thus the present research substantiates VAN OVERBEEK'S (**34**) and Muir's theories.

A study of the gross anatomy of an orchid flower will lead one to ask why the pollen has to be transferred from the anther sac to the stigmatic surface in order to be active, as the two structures lie very close to each other. The answer to this question is not known. Attempts to induce the "pollination effect," in the present work, by applying pollen and NAA to the anther sac have given negative results. It can only be suggested that some sort of interaction between pollen or auxin and the stigmatic surface is involved. While this study concerns the "pollination effect" in orchid flowers, the uniformity of the picture seems to favor the possible conclusion that this same series of physiological developments takes place in other plants.

In regard to the effect of auxin on plants in general, the results obstained in the present investigation agree with those previously reported as to the acceleration of water absorption and the aerobic nature of this phenomenon; the mobilization of material, including inorganic salts, sugars, and nitrogenous compounds; and agree with the results of some of the earlier authors on the stimulation of respiratory activity. However, the slow disappearance of starch in the ovary of the auxin-treated flower remains to be clarified. Moreover, the primary effect of auxin-stimulated respiration is more directly on water uptake than salt uptake (see the proposed chain of reactions, as postulated by COMMONER and MAZIA (**13**), COMMONER, FOGEL, and MULLER (**12**). The conclusion of these workers has been criticized by VAN OVERBEEK (**33**) who noticed a stimulation of water uptake in absence of an external supply of solutes.

Summary

An approximate two- to three-fold increase of respiration and catalase activity was demonstrated. The increase of catalase activity usually preceded the stimulation of respiration. There was no alteration of respiratory quotient. A value in the neighborhood of one was obtained, indicating the dominance of carbohydrate metabolism. The stimulation of respiration was usually apparent before there was any appreciable increase in the fresh weight of the tissue, which in turn usually preceded dry weight increase.

Following pollination, a marked increase of total nitrogen and total phosphorus (on per unit dry weight basis) in the column was observed, which was accompanied by a decrease of these substances in the perianth. On a total-per-flower basis, the phosphorus increased in one species, and decreased in another. The possibility of a re-export of material from the flower to the vegetative parts was indicated. Total sugars also increased in the developing column. The kind of sugar that underwent the greatest change varied in the two species studied. Starch that was initially present in the ovary underwent much slower digestion in the pollinated flower than in the controls. An inhibitory effect of auxin on diastase activity was suggested as a possible explanation.

The chain of reactions that happened in the column of the pollinated flower was considered to follow the order: increase in catalase activity; stimulation of respiration; acceleration of water uptake; increase of uptake of inorganic salts and mobilization of sugars; increase of hydrophilic colloids; increase in osmotic pressure.

Naphthalene-acetic and indole-acetic acids at 0.5% in lanolin applied to the stigma qualitatively duplicated the pollen effects, with NAA being more effective than IAA. Quantitatively, the auxin effect invariably ended with a leveling off, while the pollen effect showed a progressive increase. This was taken to substantiate the idea of earlier investigators that pollen does not supply the entire source of auxin necessary for continued fruit and embryo development and that more auxin is formed or released as the process proceeds.

The author wishes to express her sincere gratitude and appreciation to Dr. C. K. Schubert of Madison, Wisconsin, without whose exceptional kindness in contributing the flowers from his private orchid collection the investigation would have been impossible; to Dr. John T. Curtis for suggesting the problem and for his interest and helpful guidance throughout the course of the study; to Dr. J. F. Stauffer for his help in various ways; to Dr. W. Gordon Whaley for reading the manuscript. She is also indebted for the fellowships awarded during her studies at the University of Wisconsin.

DEPARTMENT OF BOTANY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. Bakhuyzen, H. L. Studies on Wheat Growing Under Constant Conditions. Stanford Univ. Press. 1937.
2. Bausor, S. C. Effect of growth substances on reserve starch. Bot. Gaz. **104**: 115–121. 1942.
3. Beal, J. M. Effect of indole-acetic acid on thin sections and detached segments of the second internode of the bean. Bot. Gaz. **102**: 366–372. 1940.
4. Berger, J. and Avery, G. S., Jr. Action of synthetic auxins and inhibitors on dehydrogenases of the Avena coleoptile. Amer. Jour. Bot. **30**: 297–302. 1943.
5. Berger, J. and Avery, G. S., Jr. Glutamic and isocitric acid dehydrogenases in the Avena coleoptile and the effect of auxins on these enzymes. Amer. Jour. Bot. **31**: 11–19. 1944.
6. Berger, J., Smith, P., and Avery, G. S., Jr. The influence of auxin on respiration of the Avena coleoptile. Amer. Jour. Bot. **33**: 601–604. 1946.
7. Bonner, J. The growth and respiration of the Avena coleoptile. Jour. Gen. Physiol. **20**: 1–11. 1936.
8. Bonner, J. Relations of respiration and growth in the Avena coleoptile. Amer. Jour. Bot. **36**: 429–436. 1949.
9. Brown, J. W. Effect of 2,4-dichlorophenoxyacetic acid on the water relations, the accumulation and distribution of solid matter, and the respiration of bean plants. Bot. Gaz. **107**: 332–343. 1946.
10. Brunstetter, B. C., Meyers, A. T., and Mitchell, J. W. Mineral composition of bean stems treated with 3-indole-acetic acid. Bot. Gaz. **109**: 268–276. 1948.
11. Combes, R. La nutrition azotée de la fleur. Comp. Rend. Aca. Sci. Paris. **200**: 1970–1972. 1935.
12. Commoner, B., Fogel, S., and Muller, W. H. The mechanism of auxin action. The effect of auxin on water absorption by potato tuber tissue. Amer. Jour. Bot. **30**: 23–28. 1943.
13. Commoner, B. and Mazia, D. The mechanism of auxin action. Plant Physiol. **17**: 682–685. 1942.
14. Commoner, B. and Thimann, K. V. On the relation between growth and respiration in the Avena coleoptile. Jour. Gen. Physiol. **24**: 279–296. 1941.
15. Eyster, H. C. Effect of auxins on the action of diastase in vitro. Plant Physiol. **21**: 68–74. 1946.
16. Fiske, C. H. and Subbarow, Y. The colorimetric determination of phosphorus. Jour. Biol. Chem. **66**: 375–400. 1925.
17. Gustafson, F. G. Parthenocarpy: natural and artificial. Bot. Rev. **8**: 599–654. 1942.
18. Hassid, W. Z. Determination of sugars in plants by oxidation with ferricyanide and ceric sulfate titration. Ind. & Eng. Chem., Anal. Ed. **9**: 228–229. 1937.

19. HSIANG, T. H. T. Physiological and biochemical changes accompanying pollination in orchid flowers. I. General observations and water relations. Plant Physiol. **26**: 441–455. 1951.
20. LEHMANN, O. Die quantitative Erfassung kleinster Mengen biologisch wichtiger Zuckerarten unter Ausschluss reduzierender nicht Kohlehydratartigar Körper. Planta **13**: 575–642. 1931.
21. LOOMIS, W. E. and SHULL, C. A. Methods in Plant Physiology. New York. 1937.
22. MILLER, E. C. Plant Physiology. 2nd ed. New York. 1938.
23. MITCHELL, J. E., BURRIS, R. H., and RIKER, A. J. Inhibition of respiration in plant tissues by callus stimulating substances and related chemicals. Amer. Jour. Bot. **36**: 368–378. 1949.
24. MITCHELL, J. W. Effect of naphthalene acetic acid and naphthalene actamide on nitrogenous and carbohydrate constituents of bean plants. Bot. Gaz. **101**: 688–699. 1940.
25. MITCHELL, J. W. and WHITEHEAD, M. R. Starch hydrolysis in bean leaves as affected by application of growth-regulating substances. Bot. Gaz. **102**: 393–399. 1940.
26. MUIR, R. M. Growth hormones as related to the setting and development of fruit in *Nicotiana tabacum*. Amer. Jour. Bot. **29**: 716–720. 1942.
27. MUIR, R. M. The relationship of growth hormones and fruit development. Proc. Nat. Acad. Sci. **33**: 303–312. 1947.
28. PREGL, F. Quantitative Organic Microanalysis. 3rd English ed. Philadelphia. 1937.
29. SESHAGIRIAH, K. N. Physiology of pollination in Orchidaceae. Current Science **10**: 30–32. 1947.
30. SMITH, F. G., HAMNER, C. L., and CARLSON, R. F. Changes in food reserves and respiratory capacity of bindweed tissues accompanying herbicidal action of 2,4-D. Plant Physiol. **22**: 58–65. 1947.
31. STUART, N. W. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. Bot. Gaz. **100**: 298–311. 1938.
32. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Minneapolis. 1946.
33. VAN OVERBEEK, J. Auxin, water uptake and osmotic pressure in potato tissue. Amer. Jour. Bot. **31**: 265–269. 1944.
34. VAN OVERBEEK, J., CONKLIN, M. E., and BLAKESLEE, A. F. Chemical stimulation of ovule development and its possible relation to parthenogenesis. Amer Jour. Bot. **28**: 647–656. 1941.
35. WHITE, J. The influence of pollination on the respiratory activity of the gynaecium. Ann. Bot. **21**: 487–499. 1907.
36. WOLF, B. Rapid photometric determination of total nitrogen, phosphorus and potassium in plant material. Ind. & Eng. Chem., Anal. Ed. **16**: 121–123. 1944.

CAUSES OF INJURY TO PLANTS RESULTING FROM FLOODING OF THE SOIL

PAUL J. KRAMER

(WITH FIVE FIGURES)

Received March 19, 1951

Introduction

This study was made in an effort to learn why saturating the soil with water causes almost immediate injury or death of many species of plants. While it has been shown that the deficient aeration accompanying flooding injures or kills the root systems, this fact does not explain why the shoots are injured so quickly. Injury is usually attributed to desiccation, caused by decreased water absorption through the injured roots; but this explanation seems inadequate. Plants can live for some days after the root systems are killed if the soil is kept saturated (**11**), and cut shoots can be preserved in good condition for many days in containers of water if the water is changed occasionally. Most species of plants can be grown fairly satisfactorily in shall w tanks of nutrient solution without forced aeration; but if the same species are grown in soil which is later flooded, the shoots are quickly injured. The injury of shoots cannot be caused entirely by injury to the roots as absorbing systems, because reduced absorption of water or of minerals cannot explain all of the symptoms observed in the shoots of flooded plants. Although wilting of leaves is often observed after flooding, this is not the only nor even the most characteristic symptom of injury.

Among the conspicuous symptoms of flooding injury is yellowing and death of the leaves, beginning with the lower ones and progressing up the stem. This chlorosis superficially somewhat resembles nitrogen deficiency, but often develops within four to six days after flooding, much too soon to be caused by nitrogen deficiency. The middle leaves of tomato show epinastic curvature within twenty-four to forty-eight hours after the soil is flooded; and lumps of callus tissue develop along the stem, particularly at the water level. In many species adventitious roots develop at the soil surface or just below the water surface where the water level is above the soil surface.

These occurrences cannot be explained fully as a simple result of desiccation caused by a damaged root system. Desiccation certainly would hinder the development of adventitious roots rather than stimulate their development. The epinastic curvature of leaf tips and the change in angle of petioles are also more characteristic of the reaction of turgid cells than of flaccid cells and wilted tissue. The rapid yellowing and death of the lower leaves might be caused by desiccation, or by toxic substances escaping from the dying roots or being produced in the soil which are carried upward in the transpiration stream. It, therefore, appears probable that

the various symptoms produced by flooding have several causes and are not simply the result of interference with absorption following injury to the root system.

There is extensive literature dealing with the effects of aeration on root growth and absorption, but the causes of injury by flooding are not adequately explained. The reader is referred to the monograph by CLEMENTS (5) for the earlier work and to that of the writer (13) for more recent papers.

Effects of flooding on transpiration and water absorption

A number of measurements were made of the effect of flooding on water absorption and transpiration of potted plants, mostly of Marglobe toma-

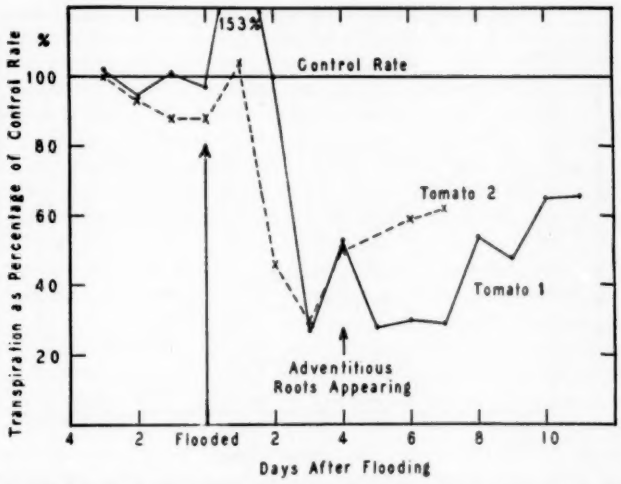


FIG. 1. Effect of flooding on transpiration of tomato plants. The values plotted are the averages of six plants. This experiment was performed in the greenhouse during the spring and summer.

toes. The plants were grown in four-inch pots of greenhouse soil until they were 15 to 18 inches high and had developed extensive root systems. For transpiration experiments, the pots were placed in metal containers and covered with oil cloth to prevent evaporation from the soil. The soil was maintained at approximately field capacity for the control plants and kept saturated by filling the containers with water for the flooded plants. Transpiration rates of two groups of tomatoes are shown in figure 1. The rate of the flooded plants is expressed as a percentage of that of the controls in order to minimize the effects of varying environmental factors on the shape of the curves. The two groups of tomatoes differed somewhat in behavior, the group flooded in the summer (group 1) showing a greater

increase in transpiration immediately after flooding, a greater decrease after a few days, and a slower and less complete recovery than the group flooded in early spring (group 2).

Several measurements were made of the effects of flooding on the capacity of the roots to absorb and conduct water under a pressure gradient. Pots containing healthy plants were placed in an open tank of water. At intervals, several pots were removed to the laboratory, the shoots cut off, and the stumps attached to a vacuum pump by a system of rubber and glass tubing. The rate of water movement through these stumps under a pressure gradient of 20 cm. of mercury was observed for hour periods. The results of two experiments are shown graphically in figure 2. The pots containing the control root systems for this experiment were soaked in water for an hour in

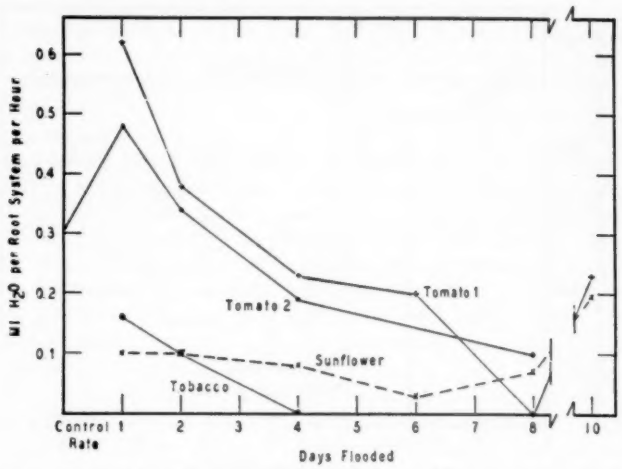


FIG. 2. Effect of flooding on water movement through detopped root systems under a pressure gradient of 20 cm. of mercury. The values for tomato 1 are the averages of eight plants, those for tomato 2, tobacco, and sunflower are the averages of three plants.

order to produce the same soil moisture conditions that existed in the flooded pots. In one experiment, sunflower, tobacco, and tomato plants were flooded and tested simultaneously to see if species differences were detectable. In all of these experiments, it was found that flooding for twenty-four hours stopped exudation from root systems not under pressure. Apparently even this short period of flooding was sufficient to produce serious injury to the roots. As shown in figure 2, large species differences were observed, tobacco roots being injured most and sunflower least severely.

In general, it appears that flooding causes a rapid decrease in the capacity of roots to absorb and conduct water, even from saturated soil. There appears to be some variation in the speed with which flooding acts, as indi-

cated by the fact that one group of tomatoes showed an increase in water intake after 24 hours of flooding while another group showed a decrease after 24 hours. The writer found that exposure of root systems to a high concentration of carbon dioxide causes an immediate reduction in water absorption (**12**), and HOAGLAND and BROYER (**9**) later reported that exposure of tomato root systems to a high concentration of carbon dioxide causes an initial decrease in permeability, followed by an increase in permeability, which they attributed to injury of the roots. Perhaps the time required to produce the initial decrease in permeability followed by an increase varies with temperature, soil conditions, or other unidentified factors. Therefore, in some experiments the period of high permeability was observed; and in others it was already past before the first measurements were made. The increase in water absorption probably occurs because the cells are killed and resistance to water movement across the root cortex is decreased. It has been observed that when root systems are killed by heat or ether that permeability to water is greatly increased for a short time (**11**). Presumably the chief reason for decrease in water absorption during a prolonged period of flooding is plugging of the conducting system. This probably is caused partly by bacterial activity in the dying roots and partly by accumulation of gum or gum-like material in the region between dead and dying tissue. The writer (**11**) found that absorption of water through root systems killed by heat or ether was quickly reduced by gum accumulation in the region between the dead and living tissue at the base of the stem. The partial recovery which was observed in a number of these experiments after a period of several days was associated in all instances with the development of adventitious roots. Tobacco root systems showed no water absorption under pressure for a period of over two weeks, but after about three weeks some adventitious roots had developed, and considerable absorption occurred.

One experiment showing the effects of flooding on transpiration of woody species is included because it shows large differences in the reaction of two species. The results are shown in figure 3. Transpiration of privet decreased rapidly after flooding, and the plants were practically dead after 12 days, while transpiration of loblolly pine seedlings was still above the control rate and was not seriously decreased until after nearly a month of flooding. This is in accord with Hunt's report (**10**) that loblolly pine is very resistant to flooding. PARKER (**18**) has also published data showing large differences among woody species in respect to tolerance to flooding.

Observations on the effects of flooding

Several species of plants growing in pots of sandy loam soil were flooded and their behavior carefully observed. The changes in appearance of the shoots following flooding are believed to be as significant as the decrease in absorption and transpiration. The experiments with herbaceous species were performed in a sunny section of the greenhouse, most of those with woody seedlings out of doors. All of these observations were made during

the summer. The pots were placed in metal tanks and tap water added until the level was one to two inches above the soil in the pots. In some experiments one set of pots was placed in a tank of water while another set of pots was buried in soil in a tank and then flooded.

Experiments with tomato

The first experiment consisted of three groups of Marglobe tomato plants about 15 inches high, growing in four-inch pots. Eight of these pots were kept in a tank of soil flooded to about 1.5 inches above the soil surface, eight pots were placed in a tank of water, and eight plants were kept watered to field capacity as controls. Symptoms of injury appeared within four days and were well developed in six days. The lower leaves were yel-

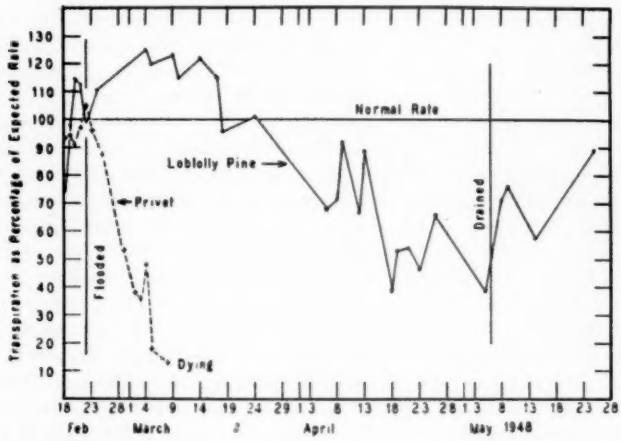


Fig. 3. Effect of flooding on transpiration of potted plants of privet (*Ligustrum japonicum* Thunb.) and loblolly pine (*Pinus taeda* L.). The values for privet are the average of three plants, those for loblolly pine the average of four plants.

low, and some were abscising; the middle and upper leaves showed definite epinastic curvature, the angle of the petioles with the stems being considerably greater than in unflooded controls. The bases of the petioles of tomato leaves usually form an angle of about 45 degrees with the stem, but those of flooded plants extended out horizontally or even drooped slightly. This is shown in figure 4. Lumps of callus tissue were visible about two thirds of the way up the stems of all the flooded plants, and considerable hypertrophy developed at the water line. The plants flooded in water were developing numerous adventitious roots, but relatively few had developed on the plants flooded in soil. After eight days of flooding, adventitious root development was much greater on plants flooded in water, and their tops showed distinctly less injury than those of plants flooded in soil. After twelve to four-

teen days of flooding, the plants flooded in water had extensive adventitious root systems, the upper leaves were in good condition, and the plants were growing and flowering. Although some adventitious roots had developed on the plants flooded in soil, these plants were in distinctly poorer condition than the plants flooded in water. The shoots of the former were not growing appreciably; the latter seemed to be partially recovering. The water in the tank where the plants were flooded in soil had a somewhat lower oxygen content than the tank of water, possibly because the organisms in the soil surrounding the pots used considerable oxygen.

This experiment was repeated with a group of younger seedlings which were growing more vigorously. In addition to eight pots immersed in water and eight pots buried in flooded soil, eight pots were immersed in Hoagland's

FIG. 4. Appearance of flooded tomato plants. The plants in the tank on the left are unflooded controls, those in the center and on the right have been flooded for five days. Note the difference in position of the petioles of the middle leaves of the flooded and unflooded plants, also the dying lower leaves.

nutrient solution. This was done to guarantee an adequate supply of nutrients and compensate for whatever leaching occurred from the pots in tap water.

These plants were flooded in the afternoon, and the next morning some plants showed inrolling of leaf tips and drooping (not wilting) of the petioles of middle leaves. After two days of flooding (with cloudy weather and showers) many leaves showed distinct epinasty, and the bases of the petioles of most of the middle leaves were at approximately right angles to the stem instead of the usual 45-degree angle. Their appearance was somewhat similar to that of plants exposed to illuminating gas (see figure 4). After three days of flooding, the lower leaves of the plants flooded in soil were turning very yellow; those of plants flooded in water and nutrient solution

were slightly less yellow. After four days the lower leaves of all flooded plants were dying, but injury was slightly greater on plants flooded in soil. Adventitious roots were developing on plants in water and nutrient solution, but not on those in the tank of soil. After a week the plants flooded in soil were definitely in worse condition than the plants flooded in water or in nutrient solution, all but the uppermost leaves being dead. Plants in nutrient solution developed slightly larger adventitious root systems than those in water, and plants flooded in soil produced relatively fewer and shorter adventitious roots. From the seventh to ninth days no further injury occurred, and the plants apparently were recovering somewhat. After nine days the pots were removed, drained, and left in the shade, where they began to recover slowly. All of the original root systems were found to have been killed, but the adventitious roots extended rapidly into the drained soil, especially on the plants flooded in water and Hoagland's solution. Apparently if the plants survive flooding long enough to develop a new adventitious root system, they will recover, though their growth has been severely checked during flooding. SARTORIS and BELCHER (**19**) mention that those varieties of sugar cane which produced few or no adventitious roots were most injured by flooding.

Simultaneously with these observations measurements were made of stem elongation and of transpiration. The results of the transpiration measurements are summarized in figure 1, group 1; the growth measurements are shown in figure 5. Growth almost ceased in a day or two after flooding but began to increase rapidly at the same time that adventitious roots began to develop. Perhaps this occurred because the new roots provided more water, but this group of plants never showed severe wilting. WENT (**22**) believes that the roots supply a hormone, caulocaline, necessary for stem growth and regards aeration as even more important for the production of this hormone than the salt absorption. If this is true, the resumption of shoot growth might be attributed to a renewed supply of hormone from the new adventitious roots.

EXPERIMENTS WITH SUNFLOWER

Pots containing sunflower plants about 50 inches in height were submerged in a tub of water. Another group of pots was buried in soil, and water was then added to a level about two inches above the level of the soil in the pots. The sunflower plants were injured relatively little as compared to tobacco and tomato. After a week one or two of the lower leaves were beginning to turn yellow, and the edges were drying out. The plants showed slight wilting the day after flooding, but did not show definite wilting again for a week, possibly because there was a little hot weather. After ten days, the plants showed relatively little injury, and the experiment was discontinued.

Adventitious roots appeared on all plants on the second or third day after flooding, and good adventitious root systems developed above the soil sur-

face after a week. There was little, if any, difference between the adventitious root systems developed on plants flooded in water and plants flooded in soil.

Another group of six plants was flooded in water through which air was bubbled. These plants were no better in appearance and produced no more adventitious roots than the plants in unaerated water. Apparently sunflower plants are less easily injured by flooding than some other species. This agrees with observations on the effect of flooding on water intake and transpiration reported earlier in this paper.

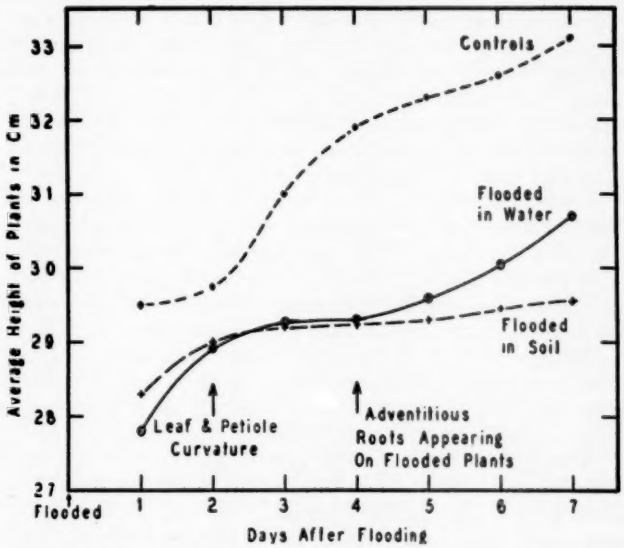


FIG. 5. Effect of flooding on stem elongation of tomato plants. Each value represents the average of eight plants.

EXPERIMENTS WITH TOBACCO

Previous experience indicated that tobacco is very sensitive to flooding, and this was found to be true in the present experiment. Plants 15 to 18 inches tall, growing in four-inch pots of greenhouse soil and in four-inch pots of sand, were flooded in tanks of water, sand, and soil. Half of these plants were potted in sand because previous experiments indicated more severe injury from flooding pots buried in soil. The weather was partly cloudy and showery during the first two days, clear and sunny the third and fourth days. In contrast to the sunflowers and tomatoes, which showed little wilting, tobacco plants were partly wilted on the second day and badly wilted after three days of flooding. In all three treatments the plants potted

in soil were more seriously wilted after three days than the plants potted in sand. The lower leaves of all of the flooded plants were dead or dying. After a week the roots and most of the submerged portion of the stems of all flooded plants were dead. Very few plants produced adventitious roots. Although the lower and middle leaves were dead, the uppermost leaves were still alive and in good condition.

Experiments with yellow poplar

Previous experience with tree seedlings indicated that flooding the soil causes serious injury to some species. Parker (**18**) found, for example, that dogwood was severely injured or killed within a week, but red, white, and overcup oak survived for several weeks. Hunt (**10**) found that loblolly pine seedlings survived in saturated soil for ten months with no visible injury to the tops, though the roots were severely injured. Yellow poplar seedlings in their third growing season were used in these experiments. The pots containing one group of seedlings were flooded in water; the pots containing another group of six seedlings were buried in soil and flooded. These plants were left out of doors. After five days one tree flooded in soil was dead, and the lower leaves of the others were dying, although none of the youngest leaves were wilted. The leaves turned yellow and then brown, and some were strongly mottled. The petioles tended to droop until they were at right angles to the stem, as with tomato. Similar symptoms existed on the trees flooded in water but to a lesser degree, and none of them were dead. After seven days all leaves on the plants flooded in soil were dead, but only half of the trees flooded in water were dead. After 12 days all the leaves of all but one tree in water were dead, and it bore only one or two living leaves.

A second set of five yellow poplar seedlings was flooded in water for two weeks. After this interval four trees were completely dead, but the upper four or five leaves of one tree were still healthy, though the lower ones were yellow and dying. The submerged portion of the stem of this tree was covered with small, white protuberances which resembled adventitious roots. This plant was transferred to a bucket of water and kept alive for about six weeks. In other experiments the lower part of the stems of several yellow poplar seedlings were enclosed in glass tubes, which were kept full of water. Considerable enlargement of the more deeply submerged portions of these stems occurred, producing an effect similar to the buttressed bases often found on cypress and tupelo gum trees growing in swamps.

Discussion

Considerable differences existed in the response of the various species to flooding. Tobacco wilted severely almost immediately, but sunflower wilted only slightly and showed little evidence of injury, while tomato wilted little or moderately, yet it showed severe injury. These results suggest that desiccation is not the only important cause of injury. The degree of injury was least in sunflower, which was the first species to develop adventitious roots,

and greatest in tobacco, which was slowest to develop adventitious roots; but it is difficult to decide which is cause and which is effect.

The data shown in figure 2 indicate that the absorption of water was reduced most rapidly and to the greatest extent in tobacco and to a lesser extent in sunflower, with reduction of tomato intermediate. It seems possible that the rapid development of adventitious roots on sunflower largely compensated for the loss of the original root system, but in tobacco the original roots all died before enough adventitious roots developed to effectively replace them.

In all experiments plants in pots buried in flooded soil suffered more injury than plants in pots standing in water. Surrounding the pots with soil prevented convection currents which might have carried some oxygen to the bottom of the tanks. Furthermore, the soil undoubtedly contained numerous microörganisms which must have used considerable oxygen. That the soil organisms play a part in causing injury is further suggested by the fact that tobacco plants potted in sand, which probably contained relatively fewer organisms than the soil, showed less injury under all conditions of flooding than plants potted in soil. Nevertheless, all plants showed injury when flooded. On the other hand, when bases of cut shoots are immersed in water, little or no injury occurs to the leaves; and adventitious roots are soon developed. This suggests that the dying root systems themselves are in some manner directly or indirectly related to the injury to the shoots.

The large reduction in water absorbing capacity of the root systems which follows flooding might seem adequate to explain the injury to the shoots. While lack of water may be an important factor in the death of the leaves, it cannot explain the curvature of the leaf tips, the change in angle of the petioles, the hypertrophy of stems at the water line, nor the development of adventitious roots. All of these occurrences are more likely to take place in turgid, growing tissue than in flaccid, wilted tissue; and they are more likely to be related to disturbance of translocation of carbohydrates and possibly of hormones than to dehydration.

Conditions are unfavorable for the translocation of organic compounds into the roots, or even below the water line. Not only is the consumption of carbohydrates in the roots greatly reduced or even ended within a few days, but the oxygen deficiency below the water line doubtless inhibits the translocation process itself. CURTIS (**6**) and MASON and PHILLIS (**15**) found that exclusion of oxygen from a portion of the stem greatly reduces the transport of carbohydrates, and DUBUY and OLSON (**7**) reported that movement of auxin in Avena coleoptiles is prevented by lack of oxygen. Interference with downward translocation probably results in accumulation of carbohydrates in the lower part of the stem, near the water line, causing hypertrophy and the development of adventitious roots. Accumulation of auxin in this region might accentuate the effects of carbohydrate accumulation on hypertrophy and root initiation. The epinastic curvature of the leaves and change in angle of the petioles of the middle leaves might also

be caused by an excess of auxin in the lower half of the stem. This change in angle was most pronounced in tomato but also occurred in yellow poplar. CHILDERS and WHITE (4) noted that the younger leaves of apple trees assumed a somewhat wider angle with the stem after the root systems were flooded, but did not discuss the cause of the phenomenon. The lower leaves of the plants in our experiments were probably too old or too quickly injured to show much response, and perhaps not enough auxin accumulated in the upper part of the stem to affect the upper leaves. There is no direct evidence to support this hypothesis, but it is an attractively simple method of explaining most of the phenomena observed to occur in stems of flooded plants. It might possibly be extended to explain the development of buttressed bases of cypress and tupelo gum trees growing in partially flooded areas. The author intends to test this hypothesis in the near future.

The yellowing and death of the lower leaves may in some instances be caused by desiccation, but it seems more likely that it is at least partly the result of poisoning by toxic substances moving up from the dying roots. These substances might be escaping from dying cells, or they might be products produced by microörganisms on the roots or in the soil. Since cut shoots of tomato can be rooted in water with little injury to the leaves, although injury occurs when the roots are left attached, it appears that the roots contribute to the injury of the shoot in some manner. Plants in pots buried in soil and flooded showed more injury than plants in pots submerged in water, and plants growing in pots of sand were injured less by flooding than those growing in pots of soil. This probably was related to decreased oxygen supply, resulting both from physical interference with oxygen movement by soil and from increased oxygen consumption by microörganisms in soil as compared to sand. Under anaerobic conditions there is much greater likelihood of the production of reduced compounds such as nitrites and sulphides which are toxic to roots and, if carried upward in sufficient quantities, might poison the leaves. It may prove difficult to ascertain the nature and role of such substances, but the problem is worthy of investigation.

Flooding probably almost completely stops the absorption of nutrients from the soil until the roots are killed. After the roots begin to die, whatever solutes are present in the soil solution are probably absorbed with the water, since the dead root cells are no longer differentially permeable. It therefore seems unlikely that deficiency of nitrogen or mineral nutrients is an important factor in injury to the shoots of flooded plants. It must be admitted, however, that the appearance of plants in flooded soil often suggests that they are suffering from mineral deficiency.

One other problem deserves mention. Although all of the roots originally present on flooded plants are usually killed, the new adventitious roots often grow vigorously. Furthermore, most of the species which are injured by flooding can be grown successfully in water culture. BERGMAN (1) found

that if the water in which the pots containing Pelargonium, Impatiens, and Phaseolus were submerged was aerated, after a week or ten days adventitious roots developed and the plants could then grow without aeration. This is another example of the difference between roots developed in soil and in water. This suggests that the roots which are produced in water are different anatomically or physiologically, or both, from roots produced in well aerated soil. It has been observed that roots produced in poorly aerated media usually contain much larger intercellular spaces than roots produced in well aerated media. BRYANT (**2**) reported that barley roots grown in unaerated cultures had more air spaces in the cortex, thinner cell walls, and a tendency to be differentiated closer to the root tips than roots in aerated cultures. SCHRAMM (**20**) found that roots of barley, corn, oats, and tomato grown in unaerated culture solutions all contained numerous large air spaces in the cortex and were less suberized than roots produced in aerated cultures. MCPHERSON (**16**) observed that more extensive development of air spaces in corn roots occurs in wet soil than in dry soil and in unaerated than in aerated culture solutions. Aeration with 50% oxygen and 50% nitrogen resulted in almost complete elimination of air spaces and excellent root growth. McPherson concluded that the air spaces were produced by collapse of cells following their death from lack of oxygen. Barley and oats were reported to behave in a similar manner, except that their oxygen requirement was lower than for corn roots.

It has generally been assumed that the air spaces result in a more adequate supply of oxygen to the cells of the roots, but the writer knows of no direct proof of this. CANNON (**3**) has presented evidence that oxygen produced by photosynthesis diffuses down to the roots of willow, and GLASSTONE (**8**) has shown that gas moves freely through the stems and roots of many plants under a slight pressure gradient. Nevertheless, it has never been demonstrated that the cells of roots with a cortex containing large air spaces are any more adequately supplied with oxygen than those in roots containing a very compact cortex. According to MCPHERSON (**16**) development of air spaces is caused by lack of oxygen, and by the time the air spaces have developed the period of most rapid metabolic activity and highest oxygen consumption probably has passed. It would seem, then, that by the time the air spaces have developed the greatest need for aeration has already passed.

It is quite possible that an important reason for survival of roots developed under water is that they are physiologically different from those produced in well aerated media. Perhaps because of differences in their respiratory enzyme systems they can actually function with a lower supply of oxygen. It would be no more difficult for a poorly aerated environment to produce differences in the physiological characteristics of roots than for it to produce the differences in structure which have been observed. Roots are physiologically different from shoots in several ways (**13**, p. 161).

Changes in enzyme systems sometimes occur during plant development. MARSH and GODDARD (**14**) and MERRY and GODDARD (**17**) found that cytochrome oxidase, which is an important enzyme in young leaves, is absent or at least not essential in the respiration of mature leaves of carrot and barley. The possibility of changes in oxygen requirement of tissue is supported by the observation of STEWARD, BERRY, and BROYER (**21**) that cells developed in water will absorb salt and respire normally at a lower oxygen concentration than cells developed in a well aerated medium.

Summary

A series of experiments was performed to learn how flooding the soil in which plants are growing causes injury or death of the shoots. Flooding is followed by a rapid reduction in transpiration and the water absorbing capacity of the roots and usually is followed by more or less wilting of the shoots. Within three or four days the lowest leaves begin to turn yellow and die, the middle leaves of tomato and yellow poplar show epinasty, and adventitious roots begin to develop on some species.

Injury to the shoots was found to be more severe when the pots were surrounded by soil and flooded than when they were simply submerged in water. Plants potted in soil were injured more than plants potted in sand. Tobacco was most injured by flooding, sunflower least, and tomato was intermediate in degree of injury. Those plants which produced adventitious roots most rapidly suffered least injury and showed the greatest degree of recovery.

While lack of water might explain the death of the leaves, it cannot explain such characteristic effects of flooding as curvature of the leaf petioles, hypertrophy of stems at the water line, nor development of adventitious roots. Flooding probably stops downward translocation of carbohydrates and auxin, and possibly their accumulation at the water line is responsible for hypertrophy and development of adventitious roots. Accumulation of auxin in the lower half of the stem might also be responsible for the epinastic curvature of the leaves and petioles. The injury and death of the leaves may be caused at least in part by toxic substances moving up from the dead roots or even from the surrounding soil.

It is believed that injury to the shoots of flooded plants is complex in origin and has several causes rather than resulting simply from interference with water absorption.

The writer wishes to acknowledge the assistance of T. T. Bannister, J. L. Hilton, F. M. Hunt, Johnson Parker, Walter Riley, and H. H. Wiebe in performing the experiments reported here. This research was supported in part by a grant from the Research Council of Duke University.

DEPARTMENT OF BOTANY
DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

LITERATURE CITED

1. BERGMAN, H. F. The relation of aeration to the growth and activity of roots and its influence on the ecesis of plants in swamps. Ann. Bot. **34**: 13–33. 1920.
2. BRYANT, A. E. Comparison of anatomical and histological differences between roots of barley grown in aerated and in non-aerated culture solutions. Plant Physiol. **9**: 389–391. 1934.
3. CANNON, W. A. Absorption of oxygen by roots when the shoot is in darkness or in light. Plant Physiol. **7**: 673–684. 1932.
4. CHILDERS, N. F. and WHITE, D. G. Influence of submersion of the roots on transpiration, apparent photosynthesis, and respiration of young apply trees. Plant Physiol. **17**: 603–618. 1942.
5. CLEMENTS, F. E. Aeration and air content. Carnegie Inst. Wash. Pub. 315. 1921.
6. CURTIS, O. F. Studies on solute translocation in plants. Experiments indicating that translocation is dependent on the activity of living cells. Amer. Jour. Bot. **16**: 154–168. 1929.
7. DUBUY, H. G. and OLSON, R. A. The relation between respiration, protoplasmic streaming, and auxin transport in the Avena coleoptile, using a polarographic microrespirometer. Amer. Jour. Bot. **27**: 401–413. 1940.
8. GLASSTONE, V. F. C. Passage of air through plants and its relation to measurement of respiration and assimilation. Amer. Jour. Bot. **29**: 156–158. 1942.
9. HOAGLAND, D. R. and BROYER, T. C. Accumulation of salt and permeability in plant cells. Jour. Gen. Physiol. **25**: 865–880. 1942.
10. HUNT, F. M. Effect of flooded soil on growth of pine seedlings. Plant Physiol. **26**: 363–368. 1951.
11. KRAMER, P. J. The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Amer. Jour. Bot. **20**: 481–492. 1933.
12. KRAMER, P. J. Causes of decreased absorption of water by plants in poorly aerated media. Amer. Jour. Bot. **27**: 216–220. 1940.
13. KRAMER, P. J. Plant and Soil Water Relationships. McGraw-Hill Book Co., New York. 1949.
14. MARSH, P. B. and GODDARD, D. R. Respiration and fermentation in the carrot, *Daucus carota*. I. Respiration. Amer. Jour. Bot. **26**: 724–728. 1939.
15. MASON, T. G. and PHILLIS, E. Further studies on transport in the cotton plant. V. Oxygen supply and the activation of diffusion. Ann. Bot. **50**: 455–500. 1936.
16. MCPHERSON, D. C. Cortical air spaces in the roots of *Zea mays*. New Phytol. **38**: 190–202. 1939.
17. MERRY, J. and GODDARD, D. R. A respiratory study of barley grain and seedlings. Proc. Rochester Acad. Sci. **8**: 28–44. 1941.

18. PARKER, J. The effects of flooding on the transpiration and survival of some southeastern forest tree species. Plant Physiol. **25**: 453–460. 1950.
19. SARTORIS, G. B. and BELCHER, B. A. The effect of flooding on flowering and survival of sugar cane. Sugar **44**: 36–39. 1949.
20. SCHRAMM, R. J. Effects of aeration on root anatomy. M.A. Thesis. Duke University. 1950.
21. STEWARD, F. C., BERRY, W. E., and BROYER, T. C. The absorption and accumulation of solutes by living cells. VIII. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. **50**: 345–366. 1936.
22. WENT, F. W. Effect of the root system on tomato stem growth. Plant Physiol. **18**: 51–65. 1943.

THE NUTRIENT-ELEMENT BALANCE OF THE TOMATO AND ITS SUSCEPTIBILITY TO *PHYTOPHTHORA INFESTANS* AS AFFECTED BY TWO LEVELS OF ZINC[1]

CYRIL B. SMITH

(WITH TWO FIGURES)

Received November 29, 1950

Introduction

Studies on the relationship between nutrition of plants and severity of diseases have indicated that nutrition is one of the factors which has a measurable effect upon the course of disease development. Since fertilizer treatments and environmental conditions affect specific host plants and pathogens differently, it is important to obtain precise knowledge of the relationships between these factors. This study deals with the relationship between zinc nutrition of the tomato and susceptibility to *Phytophthora infestans* causing Late Blight.

Most investigations on this subject (**11, 23, 24, 26, 30, 31, 32, 34, 35, 36, 37, 38**) have dealt principally with the three fertilizer elements, nitrogen, phosphorus, and potassium. In general, high nitrogen applications have increased the susceptibility of plants to disease organisms, while high potassium applications have increased the plants' resistance; but results have been inconsistent in several cases. These studies have seldom included leaf analyses so that the actual nutritional status of the plants was not taken into consideration. At times, nutrition seemed to be related to the vigor of the plants; but several other factors unquestionably were involved.

With few exceptions, the workers just cited have not concerned themselves with the micronutrient elements. There is good reason to believe that these elements play an important role in the susceptibility of a plant to a pathogen. One of the essential micronutrient elements which would seem to be very important in this relationship is zinc which is considered essential also to the growth of fungi (**12**). The zinc requirement of the fungus may be quite different from that of the host.

Little is known about the physiologic role of zinc in the plant except that zinc and copper act as a pair of mutually coordinating catalysts in oxidation–reduction reactions (**27**). It is thought that zinc plays a very important role in respiration probably by entering into the molecule of the enzyme, carbonic anhydrase, which catalyzes the reversible reaction of carbonic acid to carbon dioxide and water. This enzyme has been found in animals and was isolated from elderberry leaves by DAY and FRANKLIN

[1] Authorized for publication November 22, 1950 as Paper no. 1634 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

(5). SKOOG (25) showed that zinc was necessary for normal auxin content and that zinc could not be replaced in this function by copper or manganese. He found that a decrease in auxin preceded the appearance of visual symptoms of zinc deficiency and that a large increase in auxin occurred in the deficient plants within one to a few days after zinc was added. He concluded that zinc was not principally required for auxin synthesis but was essential for its maintenance in an active state and that lack of zinc led to excessive destruction of auxin. TSUI (33) concluded from a study of the role of zinc in auxin synthesis in the tomato that zinc was required directly for synthesis of tryptophane and indirectly for synthesis of auxins.

Compounds containing zinc are being used increasingly as fungicides. Zinc ethylene bisdithiocarbamate (Zineb) has been found effective in controlling Late Blight (*Phytophthora infestans*) and Early Blight (*Alternaria solani*) of the tomato. That the zinc is absorbed by the plant when this compound decomposes was shown by EMGE and LINN (8).

MOZNETTE *et al.* (19) reported that when pecan trees showed symptoms of rosette caused by zinc deficiency they were much more severely attacked by two fungi causing Leaf Blotch (*Mycosphaerella dendroides*) and Brown Leaf Spot (*Cercospora fusca*). When the rosetting was overcome by treatment with zinc sulphate, these diseases became less prevalent or disappeared entirely. This is a case where parasitic fungi became a minor problem when the trees were properly nourished. The leaves of pecan trees showing symptoms of rosette have been found to have a much lower zinc content than those of healthy trees (9, 10). Thus, higher zinc concentration in the leaves was correlated with resistance to disease organisms.

MILLIKAN (17) found that soil applications of zinc sulphate, especially on areas carrying the greatest infestation of root rot fungi (*Helminthosporium* spp., *Fusarium* spp., and others) resulted in very superior growth and increased yields of wheat and oats. The evidence indicated that zinc sulphate treatments assisted plants in withstanding the deleterious effects of these microorganisms, but it is not known whether this effect was due to the zinc sulphate affecting the nutritional status of the plants or to its simply acting as a fungicide.

Materials and methods

Rutgers tomato plants were grown in a greenhouse in nutrient cultures containing different levels of zinc, the concentrations of all other elements being kept constant. Since as wide a range of zinc concentrations as possible was desired, two levels of zinc were used, one as low and the other as high as possible without producing deficiency or toxicity symptoms respectively. These two critical levels were determined in a preliminary experiment.

Following the completion of the preliminary experiment, two sets of plants were produced, designated as Experiment I and Experiment II re-

spectively. Several special techniques and precautions were employed to maintain the zinc concentrations as specified. At the fruit-setting stage, the plants were inoculated with a culture of the tomato race of *Phytophthora infestans* (Mont.) deBary. Six days later, a disease index was calculated by measuring the spread of the fungus. Leaf samples were taken just prior to the time of the inoculations and analyzed for fourteen elements to determine the nutrient-element balance of the plants. The combined results were tested for statistical significance by the t test (**13**).

The nutrient culture equipment used in these experiments was specially constructed for research with micronutrient elements. In setting up this equipment, the problem of zinc contamination was considered carefully. All solutions came in contact with Pyrex glass and paraffin only, both of which were free from zinc.

Four-liter Pyrex beakers were used as containers for the nutrient solutions. Each beaker was fitted with an aerator constructed from Pyrex tubing and a Pyrex fritted disc. Both the beakers and aerators first were cleaned thoroughly with concentrated aqua regia. Water-proofed pasteboard cylinders were placed around the beakers to keep out light. Covers were prepared from twelve-inch asbestos board squares in the center of which three-inch corks with one-inch holes were placed. The complete covers were coated with high melting-point paraffin.

The same basic nutrient solution was used in all experiments, only the level of zinc being varied. The concentrations of the other elements were kept as constant as possible. A pH of 6.0 was maintained in all the solutions. A four-salt solution, commonly known as Hoagland's no. 1 nutrient solution, supplied the major elements while the micronutrient elements other than zinc were added in minute quantities by the use of a minor stock solution as described by HOAGLAND and ARNON (**14**). The composition of the basic nutrient solution was as follows (p.p.m.): N, 210; P, 31; K, 234; Ca, 200; Mg, 49; S, 64; B, 0.5; Mn, 0.5; Cu, 0.02; and Mo, 0.01. Iron was supplied as 0.5 per cent. ferrous sulphate at the rate of one milliliter per plant and was added twice weekly. Zinc was added in the various quantities required from stock solutions of zinc sulphate ($ZnSO_4 \cdot 7\,H_2O$) which contained 50 or 500 p.p.m. zinc. Cobalt was not intentionally supplied in the nutrient solutions.

Both the chemicals and water were highly purified to prevent contamination with zinc. The heavy metal impurities were removed from the four main salts by a procedure involving precipitation and absorption at a high temperature and slightly alkaline reaction in the presence of calcium and phosphate ions. This procedure and the methods for testing the purity of the salts using dithizone (diphenylthiocarbazone) were described by STOUT and ARNON (**28**). Because of the very small quantities used, salts of a chemically pure grade employed to supply the micronutrient elements were not further purified.

The water used in all experiments was redistilled in all-Pyrex glass stills.

Specially constructed glass stills designed for continuous automatic operation were used to supply the large quantities required. The solutions were made up in five-gallon Pyrex glass solution bottles, and the exact zinc content of the solution in each bottle was checked by the use of the dithizone method as described by COWLING and MILLER (3). As the plants used water, the solutions were kept at a constant level by the addition of redistilled water. The solutions were replaced entirely every two or three weeks.

The Asgrow strain of Rutgers tomato was used in all experiments. The seed was sown in washed sand in large culture dishes. After the plants were about one and one-half inches high they were transferred to Pyrex trays fitted with paraffined asbestos board covers and filled with a nutrient solution of one-half strength. None of the micronutrient elements except iron was added to this solution. When a root system had developed and the plants had grown to a height of three or four inches, they were transferred to the nutrient solution containers. The stems were wrapped with absorbent cotton and set in the corks of the covers. As the plants developed, they were pruned to a single stalk and tied to wooden stakes.

At the fruit-setting stage, just prior to the time when the inoculations were made, leaf samples were selected for chemical analyses. Since relatively large samples were required for the rather complete analyses, the basal leaves were selected. The lowest four leaves were sampled. These were cleaned by wiping with damp cheesecloth and then thoroughly dried at about 60° C after which the petioles were separated and discarded. The leaf blades were finely ground using a large porcelain mortar and pestle in order to avoid mineral contamination. These ground samples were dried further at 100° C for several hours before being weighed out for the determinations.

The ground leaf blade samples were analyzed for fourteen elements. A systematic procedure using a 7.5-gram sample was employed for the elements phosphorus, potassium, calcium, magnesium, sodium, sulphur, iron, manganese, zinc, copper, molybdenum, and cobalt, while two 0.5-gram samples were used for the boron and nitrogen determinations respectively. All these determinations were done in duplicate or triplicate. The systematic procedure used was essentially that of PARKS *et al.* (20), but several modifications were made. In the preparation of solution A, a dry ashing procedure was adopted. The methods of ELLIS and THOMPSON (7) and DROSDOFF and NEARPASS (6) were substituted in the determinations of cobalt and magnesium respectively while a flame photometer was used in the determinations of potassium, calcium, and sodium. Boron was determined by the method of AUSTIN and MCHARGUE (1) and the Winkler modification of the Kjeldahl-Gunning method was used to determine total nitrogen (22).

Before making the inoculations, the entire nutrient culture assembly was enclosed in a clear parafilm tent which was moistureproof but allowed light to pass through. The humidity was raised to saturation by the use of a humidifying unit. The inoculations were made in the evening so that infec-

tion would take place during the night when the temperature was about 60° F. The greenhouse was also kept as cool as possible during the day. Fifteen leaflets on three fully-matured leaves were inoculated on each plant. Usually the fifth, sixth, and seventh leaves were selected.

Cultures of the tomato race of *Phytophthora infestans* were produced in test tubes on lima-bean agar, the preparation of which was described by CLINTON (**2**). These tubes were kept in a constant temperature chamber at 18° C for about ten days. The sporangia were then washed out of the tubes with redistilled water and germinated in small culture dishes by placing them in a constant temperature chamber at 12° C for about three hours, at which time the mobile swarm spores could be seen under the microscope. These optimum conditions for the production and germination of sporangia were determined by CROSIER (**4**). A diluted suspension of the swarm spores was used for the inoculations. Small pieces of absorbent cotton were dipped into this suspension and attached to the tips of the leaflets. After six days the progress of the late blight fungus was determined by measuring the spread of the necrosis from the absorbent cotton towards the base of the leaflet. The length of the lesion along the main vein of the leaflet in millimeters was taken as a disease index as suggested by MILLS (**18**).

To determine the range of zinc concentrations at which normal plants could be produced, a preliminary experiment was conducted in which tomatoes were grown at twelve levels of zinc, varying from 0 to 25 p.p.m. as shown in table I. These plants were grown between March 5 and May 14, 1948. When the normal plants had reached the fruit-setting stage, leaf samples were taken from plants grown at seven of the levels. Since only

TABLE I

APPEARANCE OF PLANTS AND CORRESPONDING ZINC CONTENT OF THE LEAFLETS IN THE PRELIMINARY EXPERIMENT

	Levels of zinc in nutrient solutions	Symptoms	Zinc content
	p.p.m.		*γ/g.* dry weight
1.	0.0	Severe necrosis and very stunted	9
2.	0.005	Some necrosis and stunted	....
3.	0.01	No necrosis but slightly stunted	15
4.	0.025	Normal	65
5.	0.05	Normal	....
6.	0.1	Normal	....
7.	0.5	Normal	86
8.	1.0	Normal	....
9.	2.5	Normal	198
10.	5.0	Slight chlorosis but not stunted	526
11.	10.0	Chlorosis and stunted	833
12.	25.0	Severe chlorosis and very stunted	1489

two plants were grown at each level, composite samples of the basal and median leaves were selected. The plants grown at 0 p.p.m. made such limited growth that small leaf samples were collected at the end of the second week's growth. These samples were analyzed for zinc only.

In Experiment I, tomato plants were grown at the two critical levels of zinc selected in the preliminary experiment, namely, 0.025 and 2.5 p.p.m. There were thus two zinc treatments with six plants per treatment and four replications, making a total of 48 plants. The treatments were randomized within each replication to minimize the influence of any differences in light or temperature. This set was produced between September 18, and December 16, 1948. In Experiment II, the plants were grown and treated in exactly the same manner as in Experiment I. This set was produced between December 26, 1948, and March 28, 1949.

Experimental results

Preliminary experiment

The plants grown at 12 levels of zinc between 0 and 25 p.p.m. showed wide variations in appearance and in leaf zinc concentration as presented in table I. The plants produced at concentrations between 0.025 and 2.5 p.p.m. appeared completely normal at the fruit-setting stage. As the concentration was lowered from 0.025 to 0 p.p.m., an increasing degree of stunting and necrosis occurred. These symptoms of zinc deficiency agreed with those noted in other investigations (**25, 29, 33**), the cessation of growth being evident first, followed by development of brown necrotic areas on the leaves. There was an increasing degree of stunting and chlorosis at the other extreme as the concentration was raised from 2.5 to 25 p.p.m. The plants produced in the cultures to which no zinc was added developed to a height of six inches only. The necrotic areas spread until the plants were completely killed about three weeks after being set in the deficient solution. The plants produced at 25 p.p.m. were badly stunted and very chlorotic but had not been killed at the time the experiment was terminated.

It is observed in table I that there was a very large increase in the zinc concentration of the leaflets as the zinc concentration in the solutions was increased. This varied from 9 γ/g. in the deficient solutions to 1489 γ/g. in the solutions containing 25 p.p.m. which was a 165-fold difference. The zinc concentrations in the normal leaflets varied from 65 to 198 γ/g.

Experiment I

In this experiment the disease indices of the low zinc treatment were consistently higher than those of the high zinc treatment as shown in table II. The treatment mean (49.2 mm.) for the low zinc treatment was about 10% higher than that for the high zinc treatment (44.3 mm.).

The results of the chemical analyses (means of values from four replications) are represented in histograms in figure 1. The zinc concentration in the leaflets of the high zinc treatment is about triple that in the low zinc

TABLE II

SPREAD OF THE LATE BLIGHT FUNGUS (IN MILLIMETERS) SIX DAYS AFTER INOCULATION

Treatment	Replication	Experiment I	Experiment II
1. Low zinc level (0.025 p.p.m.)	1	52.2[1]	27.2
	2	49.0	32.8
	3	48.4	35.0
	4	47.0	33.6
	Mean	49.2	32.2
2. High zinc level (2.5 p.p.m.)	1	45.0	31.5
	2	40.5	33.3
	3	44.4	30.3
	4	47.3	31.2
	Mean	44.3	31.6

[1] Values are means of from 72 to 90 measurements on six plants.

treatment. Considerable differences are noted in the concentrations of the other elements. Manganese and molybdenum are conspicuous in that their concentrations in the high zinc treatment are nearly double those of the low zinc treatment. Nitrogen is 10% higher in the high zinc treatment, phosphorus 29%, potassium 3%, and sulphate 14%. In contrast, sodium is 28% higher in the low zinc treatment, boron 21%, and iron 9%. The concentrations of calcium, magnesium, copper, and cobalt are about equal in both treatments.

Experiment II

In this experiment there were no consistent differences in the disease indices between the two treatments as shown in table II.

The results of the chemical analyses are represented in histograms in figure 2. As in Experiment I, it is noted that the zinc concentration in the

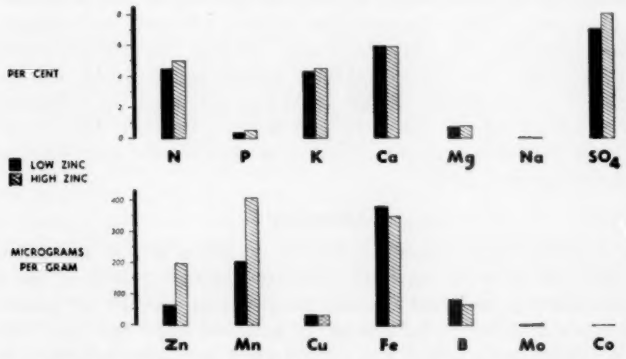


Fig. 1. Histograms illustrating the effects of the two zinc levels on chemical composition of tomato leaflets in Experiment I.

leaflets of the high zinc treatment is about triple that in the low zinc treatment. Considerable differences can again be seen in the concentration of the other elements. Nitrogen is 19% higher in the high zinc treatment, potassium 4%, sodium 13%, sulphate 11%, manganese 26%, molybdenum 70%, and cobalt 18%. In contrast, phosphorus is 19% higher in the low zinc treatment, calcium 3%, and copper 24%. The concentrations of magnesium, boron, and iron remained at about equal concentrations in each treatment.

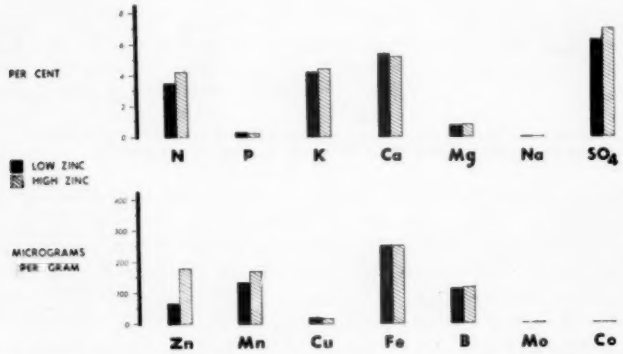


FIG. 2. Histograms illustrating the effects of the two zinc levels on chemical composition of tomato leaflets in Experiment II.

COMBINED RESULTS

Calculated *t* test values on the combined results of Experiments I and II are shown in table III. The statistical analyses indicate that when the experiments are combined, there are highly significant differences between the concentrations of elements in the leaflets of plants produced at the low and high zinc levels. The concentrations of zinc, nitrogen, and sulphate are greater at the high zinc level than at the low zinc level and are significant at the 1% point. The concentrations of potassium, manganese, and molybdenum also are significantly higher at the high zinc level, the differences being well beyond the 5% point. In both experiments, all of the plants were completely normal in appearance and at no time showed any symptoms of zinc deficiency or toxicity.

Discussion

In the preliminary experiment, distinct symptoms of both zinc deficiency and zinc toxicity were obtained. The very limited growth of the zinc-deficient plants showed that the selection of nutrient culture equipment and the methods used for the purification of salts and water had been satisfactory in eliminating traces of zinc. Since there were clear-cut limits in the concentrations at which normal plants were produced, these two limits, 0.025

and 2.5 p.p.m. were used in subsequent experiments. There was thus a 100-fold difference in zinc concentration between the low and high levels which resulted in a threefold difference in leaf zinc concentration. Considering the complete range of concentrations of a 2500-fold increase of zinc in the solutions produced a 100-fold increase in the leaves.

In both experiments the zinc concentration in the leaflets of the high zinc treatment was about triple that in the low zinc treatment. Thus, as in the preliminary experiment, a 100-fold difference in the zinc levels of the nutrient cultures resulted in a threefold difference in leaf concentration of zinc, although the absolute differences were relatively small.

TABLE III

TREATMENT MEANS FOR EACH EXPERIMENT AND CORRESPONDING T TEST VALUES

		Experiment I		Experiment II		T test values
		Low zinc level	High zinc level	Low zinc level	High zinc level	
Disease index (mm.)		49.2	44.3	32.2	31.6	1.79
N	(%)	4.50	4.97	3.51	4.16	6.63*
P	"	.35	.45	.32	.27	.93
K	"	4.34	4.47	4.15	4.32	3.20†
Ca	"	5.98	5.94	5.35	5.17	1.69
Mg	"	.75	.78	.73	.75	1.27
Na	"	.044	.034	.039	.044	.74
SO_4	"	7.09	8.05	6.31	7.01	4.63*
Zn	(γ/g.)	64	198	67	180	16.47*
Mn	"	206	408	133	168	3.49†
Cu	"	32	31	21	17	.78
Fe	"	381	348	252	251	1.64
B	"	82	68	111	117	.56
Mo	"	2.40	4.69	.76	1.29	3.35†
Co	"	.20	.26	.22	.21	1.13

*Significant at 1 per cent. point.
†Significant at 5 per cent. point.

In Experiment I where the disease indices were about 10% higher under the low zinc treatment than in the high zinc treatment, the difference in zinc concentration of the leaflets between the two treatments is only slightly less than in Experiment II where no significant differences in the disease indices were obtained. It is noted that the differences in concentration of certain other elements especially manganese and molybdenum are much higher where the disease indices are lower. The generally lower disease indices in Experiment II can be explained by the less favorable inoculation conditions especially higher temperatures in March in contrast to the more favorable conditions in December when the plants of Experiment I were inoculated.

While the differences in the disease indices between the low and high zinc treatments cannot be considered significant, the results do indicate a trend. In Experiment I, the indices in the low zinc treatment were consistently higher than those of the high zinc treatment. While the magnitude of these

differences was not duplicated in Experiment II, there were slight differences in the same direction. It should be noted that the disease index for each plant was the mean length of the lesions on 12 to 15 leaflets.

Several differences have been noted between the leaf compositions in Experiment I compared with Experiment II. These are probably the result of differences in light in the greenhouse since Experiment I was conducted when the days were continually becoming shorter while in Experiment II, the days were continually lengthening as the experiment progressed.

In general, the same trends were observed in both experiments with respect to the two treatments lowering or raising the concentration of an element but phosphorus, sodium and boron were exceptions. For example, phosphorus absorption increased in Experiment I but decreased in Experiment II at the high zinc level. These differences in absorption cannot be explained by differences in pH since this was kept constant at pH 6.0.

The chemical analyses presented here do not agree closely with other rather complete analyses made by PARKS *et al.* (**21**), LYON *et al.* (**16**), or LEWIS and MARMOY (**15**). In the first two investigations the tomatoes were produced in nutrient cultures, the composition of which was quite different from that used in these studies. In the latter investigation the plants were produced in soil. These analyses are thus not comparable.

When the t test was applied to the results of both experiments, it was noted that the values for zinc, nitrogen, and sulphate were well beyond the 1% point value while the values for potassium, manganese, and molybdenum were much higher than the 5% point and approached the 1% point values. The over-all differences in these six elements between the low zinc and high zinc treatments can therefore be considered highly significant statistically.

Thus, producing tomatoes at as low and as high zinc levels as possible without producing plants abnormal in appearance has resulted in marked differences in chemical composition; but no significant differences in disease susceptibility has resulted, although a trend has been indicated. The much higher zinc concentrations of the leaflets in the high zinc treatment has influenced the nutrient-element balance of the plants by raising the concentrations of nitrogen, potassium, sulphate, manganese, and molybdenum significantly.

Summary

In this study, Rutgers tomato plants were grown in a greenhouse in rigidly controlled nutrient solutions at two levels of zinc, namely, 0.025 and 2.5 p.p.m. These two levels had been selected in a preliminary experiment as being as low and as high as possible without producing deficiency or toxicity symptoms respectively.

The susceptibility of the plants to the Late Blight fungus was determined by inoculating them at the fruit-setting stage with a culture of the tomato race of *Phytophthora infestans* (Mont.) deBary and calculating disease indices. Just before the inoculations were made, samples of basal leaves were

selected and the leaflets were analyzed for fourteen nutritionally important elements to determine the nutrient-element balance of the plant.

The variation in the zinc levels resulted in no statistically significant effects on disease susceptibility; but the trend was definitely towards greater resistance to the Late Blight fungus at the higher zinc concentration.

Although the plants appeared completely normal, the zinc concentrations in the leaflets of the high zinc treatment were consistently about triple those in the low zinc treatment.

The large increase in the zinc concentration of the leaflets produced at the high zinc level influenced the nutrient-element balance by significantly raising the concentrations of nitrogen, potassium, sulphate, manganese, and molybdenum in the leaflets.

The writer gratefully acknowledges the generous advice and assistance of Dr. Walter Thomas, Dr. Warren B. Mack, Miss Marguerite Desaulniers and Mr. George A. Taylor of the Department of Horticulture and Dr. W. R. Mills of the Department of Botany.

Department of Horticulture
Pennsylvania State College
State College, Pennsylvania

LITERATURE CITED

1. Austin, C. M. and McHargue, J. S. The determination of total boron in plant materials with "chromotrope-B." Jour. Assoc. Off. Agr. Chem. **31**: 427–431. 1948.
2. Clinton, G. P. Report of the botanist for 1908. IV. Artificial cultures of phytophthora, with special reference to oospores. Connecticut Agr. Exp. Sta. Ann. Rpt., pp. 891–907. 1908.
3. Cowling, H. and Miller, E. J. Determination of small amounts of zinc in plant materials. Ind. & Eng. Chem., Anal. Ed. **13**: 145–149. 1941.
4. Crosier, W. Studies in the biology of *Phytophthora infestans* (Mont.) deBary. Cornell Memoir **155**. 1934.
5. Day, R. and Franklin, J. Plant carbonic anhydrase. Science N.S. **104**: 363–365. 1946.
6. Drosdoff, M. and Nearpass, D. C. Quantitative micro-determination of magnesium in plant tissue and soil extracts. Anal. Chem. **20**: 673–674. 1948.
7. Ellis, G. H. and Thompson, J. F. Determination of cobalt in biological materials with nitrosocresol. Ind. & Eng. Chem., Anal. Ed. **17**: 254-257. 1945.
8. Emge, R. G. and Linn, M. B. The effect of zinc ethylene bisdithiocarbamate on the growth responses of the tomato plant. Phytopath. **40**: 8. 1950 (Abstract).
9. Finch, A. H. Zinc and other mineral constituents in relation to the rosette disease of pecan trees. Jour. Agr. Res. **52**: 363–376. 1936.

10. Finch, A. H. and Kinnison, A. F. Pecan rosette: soil, chemical and physiological studies. Arizona Agr. Exp. Sta. Tech. Bull. **47**: 407–442. 1933.
11. Fisher, P. L. Physiological studies on the pathogenicity of *Fusarium lycopersici* Sacc. for the tomato plant. Maryland Agr. Exp. Sta. Bull. **374**. 1935.
12. Foster, J. W. The heavy metal nutrition of fungi. Bot. Rev. **5**: 207–239. 1939.
13. Goulden, C. H. Methods of statistical analysis. John Wiley and Sons. 1939.
14. Hoagland, D. R. and Arnon, D. I. The water-culture method for growing plants without soil. California Agr. Exp. Sta. Cir. **347**. 1939.
15. Lewis, A. H. and Marmoy, F. B. Nutrient uptake by the tomato plant. Jour. Pom. Hort. Sci. **17**: 275–283. 1939.
16. Lyon, C. B., Beeson, K. C. and Ellis, G. H. Effect of micro-nutrient deficiencies on growth and vitamin content of the tomato. Bot. Gaz. **104**: 495–514. 1943.
17. Millikan, C. R. A preliminary note on the relation of zinc to disease in cereals. Jour. Agr. Victoria, Australia **36**: 409–416. 1938.
18. Mills, W. R. The influence of maturity of potato varieties upon their susceptibility to late blight. Amer. Pot. Jour. **15**: 318–325. 1938.
19. Moznette, G. F. Insects and diseases of the pecan and their control. U. S. Dept. Agr. Farmers Bull. 1829: 44–48. 1940.
20. Parks, R. Q., Hood, S. L., Hurwitz, C., and Ellis, G. H. Quantitative chemical microdetermination of twelve elements in plant tissue. Ind. & Eng. Chem., Anal. Ed. **15**: 527–533. 1943.
21. Parks, R. Q., Lyon, C. B., and Hood, S. L. Some effects of boron supply on the chemical composition of tomato leaflets. Plant Physiol. **19**: 404–419. 1944.
22. Piper, C. S. Soil and Plant Analysis. Interscience Publishers. 1944.
23. Roberts, F. M. Factors influencing infection of the tomato by *Verticillium albo-atrum*. Ann. Appl. Biol. **30**: 327–331. 1943.
24. Shear, G. M. and Wingard, S. A. Some ways by which nutrition may affect severity of disease in plants. Phytopath. **34**: 603–605. 1944.
25. Skoog, F. Relationships between zinc and auxin in the growth of higher plants. Amer. Jour. Bot. **27**: 939–951. 1940.
26. Spencer, E. L. and McNew, G. L. The influence of mineral nutrition on the reaction of sweet corn seedlings to *Phytomonas stewarti*. Phytopath. **28**: 213–223. 1938.
27. Stiles, W. Trace Elements in Plants and Animals. Cambridge. 1946.
28. Stout, P. R. and Arnon, D. I. Experimental methods for the study of the role of copper, manganese and zinc in the nutrition of higher plants. Amer. Jour. Bot. **26**: 144–149. 1939.

29. STOUT, P. R., OVERSTREET, R., JACOBSON, L. and ULRICH, A. The use of radioactive tracers in plant nutrition studies. Proc. Soil Sci. Soc. Amer. **12**: 91–97. 1947.
30. THOMAS, H. R. Effect of nitrogen, phosphorus, and potassium on susceptibility of tomatoes to *Alternaria solani*. Jour. Agr. Res. **76**: 289–306. 1948.
31. THOMAS, W. and MACK, W. B. A foliar diagnosis study of the nutrition of greenhouse tomatoes in relation to the incidence of a disease. Pennsylvania Agr. Exp. Sta. Bull. **405**. 1941.
32. THOMAS, W. and MACK, W. B. Susceptibility to disease in relation to plant nutrition. Science N.S. **93**: 188–189. 1941.
33. TSUI, C. The role of zinc in auxin synthesis in the tomato plant. Amer. Jour. Bot. **35**: 172-179. 1948.
34. WALKER, J. C. Soil management and plant nutrition in relation to disease development. Soil Sci. **61**: 47–54. 1946.
35. WALKER, J. C. and FOSTER, R. E. Plant nutrition in relation to disease develoment. III. Fusarium wilt of tomato. Amer. Jour. Bot. **33**: 259–264. 1946.
36. WALKER, J. C. and HOOKER, W. J. Plant nutrition in relation to disease development. I. Cabbage yellows. Amer. Jour. Bot. **32**: 314–320. 1945.
37. WALKER, J. C. Plant nutrition in relation to disease development. II. Cabbage clubroot. Amer. Jour. Bot. **32**: 487–490. 1945.
38. WINGARD, S. A. The nature of disease resistance in plants. Bot. Rev. **7**: 76–87. 1941.

THE EFFECTS OF THE FORM OF NITROGEN AND THE AMOUNT OF BASE SUPPLY ON THE ORGANIC ACIDS OF TUNG LEAVES

SEYMOUR G. GILBERT, CORNELIUS B. SHEAR AND CLARE M. GROPP

Received February 13, 1951

Recent research (**10**) on the nutrition of the tung plant has demonstrated a close relationship betwen cation uptake and the form of nitrogen supplied. For example, tung seedlings grown with all or part of the nitrogen supplied as the ammonium salt have a lower calcium requirement for optimal growth than do those supplied with only nitrate as the nitrogen source. It has been demonstrated that a close relationship exists in the tung plant between the growth of the tree and the balance of the cations supplied (**9**). It was the purpose of the present investigation to determine if the form of nitrogen and ratios of cations supplied affect the production of anions within the plant, particularly the organic acids.

Methods

Oven-dried, midshoot leaves were available from the 1947 experiments on nutrient balance of tung (**10**). These one year old seedlings had been grown in sand culture in a factorial experiment with three levels of Mg and K (0.5, 2, and 4 millimoles/l.) and three ratios of nitrate N to ammonium N (all NO_3; $\frac{3}{4}$ NO_3 to $\frac{1}{4}$ NH_4; $\frac{1}{2}$ NO_3 to $\frac{1}{2}$ NH_4). In a supplementary experiment, the highest levels of K and Mg of the main experiment were supplied with three levels of calcium (0.4, 2, and 5.7 millimoles/l.) in an all nitrate solution, and also in a $\frac{1}{2}$ NO_3 to $\frac{1}{2}$ NH_4 solution. Thus the material represented plants grown under a wide range of cations and of forms of nitrogen supply.

Comparable leaves from these trees had been previously analyzed for mineral content, and determinations for total organic acid and oxalic acid content were made essentially as described by PUCHER *et al.* (**7, 8**), using a Goldfisch extraction apparatus and a Beckman pH meter calibrated at pH 2.6 and 8.0 with appropriate standard buffers. It was found that an organic acid fraction that deteriorated rapidly in alkaline solution was present in the ether extract of tung leaves. This necessitated keeping the extract in ether until within an hour of the time the titrations were made. The ether extract was made alkaline, then the ether was quickly evaporated, the solution cooled, made acid, and the titrations made immediately after adjustment to pH 8.0.

The data obtained were calculated as milliequivalents of H per gram of dried leaf, and the difference between the total organic acids by titration and the oxalic acid by permanganate oxidation was calculated as non-oxalic acid fraction. The data for the total and non-oxalic acid fractions are

purely comparative since a proper correction factor (7) was not established for the unknown acids. Oxalic acid was isolated from tung leaves and identified by the formation and melting points of the monohydrate and *p*-toluidide. The relationships between these three fractions and the treatments supplied the plants were examined in two ways. The organic acid data were tested by analysis of variance for the main effects and interactions of the factorial treatments in the main and supplementary experiments. Multiple regression (11) was used to test for relationships between the organic acid fraction and the K, Ca, Mg, and N contents of the leaves from the combined experiments.

Results

The data in table I show that decreasing the ratio of nitrate to ammonium ions progressively lowers the oxalic acid content with the lowest level of this acid being found in the leaves of the $\frac{1}{2}$ ammonium treatment. The corresponding significant relation of N source to accumulation of non-oxalic acids is related to the change from all nitrate to $\frac{1}{4}$ ammonium, additional ammonium being without significant effect. These additive effects of N source on the acid fractions are reflected in the data for total acids which show a progressive decrease in acids with each increase in ammonium. The high F value of 64.3 supporting the oxalic acid response shows the relation to be very pronounced. This is also reflected in the F value of 76.1 for total acids.

TABLE I

MAIN EFFECTS OF N SOURCE AND K, MG, AND CA LEVEL ON ORGANIC ACID CONTENT OF TUNG LEAVES

	Organic acid fraction m.e./gm. dry leaf		
	Total	Oxalic	Non-oxalic
NO_3	1.87	0.77	1.08
0.75 NO_3, 0.25 NH_4	1.61	0.67	0.94
0.5 NO_3, 0.5 NH_4	1.39	0.46	0.95
K_1, 0.5 millimoles/l.	1.61	0.63	1.01
K_2, 2.4 millimoles/l.	1.66	0.71	0.94
K_3, 4.0 millimoles/l.	1.59	0.58	1.02
Mg_1, 0.5 millimoles/l.	1.62	0.67	0.98
Mg_2, 2.4 millimoles/l.	1.67	0.70	0.96
Mg_3, 4.0 millimoles/l.	1.57	0.54	1.03
L.S.D.* .05	0.08	0.06	0.09
Ca_1, 0.4 millimoles/l.	1.28	0.49	0.79
Ca_2, 2.0 millimoles/l.	1.52	0.42	1.10
Ca_3, 5.7 millimoles/l.	1.71	0.95	0.76
L.S.D.** .05	0.31	0.06	0.11

* Average of 36 plants.
** Average of 12 plants.

TABLE II

INTERACTION OF N AND CA ON OXALIC ACID CONTENT OF TUNG LEAVES IN M.E. ACID/GM. DRY LEAF

Source of N	Ca level			
	1	2	3	Average
1 All NO_3	0.72	0.51	1.14	0.79
2 ½NO_3, ½NH_4	0.26	0.33	0.75	0.45
Average	0.49	0.42	0.95	....

F of interaction is 13.3; F required at 0.01 is 8.7.

The K effect (table I) is significant for the oxalic data only and the highest oxalic acid content is found at K_2, the differences from K_1 and K_3 being highly significant. The data in table I show that the only significant Mg difference in oxalic acid occurs between the two lowest and the highest levels. Since the Mg effect on non-oxalic acids is not significant, the Mg effect on total acids is largely the result of its effect on oxalic acid.

The above effects of N source on acid production were also evident in the data from the N × Ca supplementary experiment. The Ca effect, unlike those of K and Mg, is outstanding. This is especially true for the oxalic acid content. In this case the Ca_3 treatment produced nearly twice as much oxalic acid as the Ca_1 treatment. A highly significant N × Ca interaction is found in these data. Examination of the interaction in table II shows that the average drop in oxalic acid content of 0.07 unit from Ca_1 to Ca_2 for both sources of N is the net result of a drop of 0.21 unit between these two Ca levels at N_1 with a corresponding rise of 0.07 unit at N_2.

A multiple correlation analysis between organic acid fractions and concentrations of mineral nutrients in the leaves provided the statistics set

TABLE III

MULTIPLE REGRESSION OF NUTRIENT CONTENT OF THE LEAF ON ORGANIC ACID FRACTION CONTENT

Nutrient	Acjd fraction					
	Total		Oxalic		Non-oxalic	
	b'*	r**	b'	r	b'	r
K	−.14	.12	.02	.16	−.29	−.04
Ca	.31	.72	.54	.73	−.14	.27
Mg	−.17	.07	.02	.01	−.35	.05
N	−.72	−.79	−.35	−.65	−.66	−.39
R (multiple correlation coefficients	.87		.80		.46	
Required for sig.	r	R				
at .05	.35	.51				
.01	.45	.59				

* b' = Standard partial regression coefficient.
** r = Correlation coefficient.

forth in table III. The standard partial regression coefficients show that the uptake of calcium was positively and the nitrogen was negatively related to the oxalic acid content. Potassium and magnesium were not closely related to oxalic acid content. The simple correlation coefficients show that calcium and nitrogen are related to the non-oxalic acid fraction in the same manner as for the oxalic acid fraction, but the relationships are not so close. There was very little association between non-oxalic acid fractions and the potassium and magnesium content.

Individual regression data are given for each of the three nitrogen sources in table IV. Nitrogen content increased with increasing ammonium

TABLE IV

CO-VARIANCE OF NITROGEN SOURCE WITH NIGROGEN AND OXALIC ACID CONTENTS OF TUNG LEAVES

Source	Average % N	Regression coefficient b	Standard deviation Sb
All NO_3	3.01	0.59	0.34
$\frac{3}{4}NO_3$, $\frac{1}{4}NH_4$	3.53	0.09	0.23
$\frac{1}{2}NO_3$, $\frac{1}{2}NH_4$	3.98	−.13	0.24
F obtained	105.0		
F at .05	3.33		
.01	5.42		

in the solution presumably because of increased uptake of ammonium nitrogen with increasing concentration of this form. This highly significant effect is responsible for the overall negative regression of −0.13 of oxalic acid on nitrogen which is increased to a negative regression of −0.35 when nitrogen is adjusted for the potassium, magnesium, and calcium content by means of a multiple regression.

Discussion

The older literature (**3, 4**) showed that plant species differ widely in both oxalic acid content and calcium oxalate formation. Various conflicting theories based on too little quantitative evidence were offered to explain these differences (**4**). The more recent quantitative methods (**7, 8**) have produced two generally accepted conclusions: first, that with all nitrate supply more organic acid production occurs than with part or all ammonium supply (**5, 13, 14**); and, second, that cation content is directly correlated with organic acid content (**6, 12**). A recent paper (**1**) attempts to relate these two conclusions by determining whether the effects of different N sources on organic acids of cotton are produced as part of the difference in N assimilation or as the result of their different ionic properties as affecting cation-anion balance. Only the source of nitrogen was varied, the effect on cation being measured by effect on intake from a constant supply. While these authors (**1**) favor the latter hypothesis, they do not regard their own evidence from cotton as conclusive. Unfortunately, for comparison with the

present paper, their data show that oxalic acid does not play a quantitatively important part in the metabolism of the cotton plant, since that plant contains about 4% of oxalic acid in a total of 2.76 m.e. per gram, while tung contains about 41% in a total of 1.87 m.e.

The experiments on tung provide considerable information on effects of nitrogen source and on amounts and ratios of bases. The relation of the organic acids to cation balance in tung has been discussed elsewhere (**9**). Attention will be directed here to the metabolism associated with the production of organic acids in response to nutritional variables in a plant with the ability to form relatively high amounts of oxalic acid.

This study can be interpreted by the hypothesis that in the tung plant the reduction of nitrate nitrogen to the amino state by oxidation of sugar results in the formation of organic acids, including oxalic, as by-products of the metabolic process. Oxalic acid in particular can be regarded as a terminal product resulting from a high oxidizing potential of nitrate reduction by carbohydrate oxidation rather than as an intermediate in complete aerobic respiration. The accumulation of oxalic acid as a by-product is favored by the intake of Ca, with the formation of insoluble calcium oxalate. Thus oxalic acid formation in tung is directly proportional to both calcium supply and calcium uptake. The latter is influenced by the other bases (**9**) which may consequently indirectly influence oxalic acid formation. In these plants, both K and Mg lowered the Ca content of the leaves (**10**) with all sources of N, the effects being most pronounced with all nitrate treatments.

When part of the nitrogen is supplied to the plant as the ammonium form, the resulting lessened accumulation of oxalic acid may result from decreased formation of oxalic acid as a terminal product of carbohydrate oxidation with nitrate reduction. Amination and amide formation of labile intermediates would also help lower the organic acid formed with the respiratory processes. Increasing the NH_4 ratio, however, does not produce as marked an effect on the non-oxalic acids as on oxalic. This indicates that the major effect of the ammonium may be a lessened formation of oxalic acid, rather than an increased utilization. The role of calcium may be explained on the basis of the decreased uptake of Ca with NH_4 being either a result or a cause of the lowered oxalate content.

A connection can be established between these data and an experiment (**6**) on oats in which the nitrogen and oxygen supply to the roots were varied. Here, likewise, the nitrate ion was specifically identified with organic acid accumulation, both of oxalic and non-oxalic acids. The production of oxalic acid in plus nitrate culture, while high in low oxygen cultures, was markedly inhibited by high oxygen tension. The minus nitrate cultures had a very low content of oxalic acid at all levels of oxygen. Thus oxalic acid formation may be the result of oxidation by nitrate in the anaerobic phase of respiration. This is in keeping with further data (**2**) on these plants, which showed that nitrate reduction was likewise inhibited at high oxygen tension and accelerated at low oxygen tensions. Thus nitrate reduction was a necessary part of oxalic acid formation.

It can be concluded that, under conditions of nitrate supply, a substantial part of the calcium intake of the tung plant is precipitated when oxalate is formed as a by-product of nitrate reduction. As is shown in this and related experiments (**9, 10**), the calcium requirement of high-ammonium tung plants is lower than in those given high nitrate ratios. The increasing use of anhydrous ammonia as a source of nitrogen in fertilizer formulation in preference to nitrate, therefore, will require re-examination of the calcium availability and requirement of plants, such as tung, that accumulate calcium oxalate under nitrate metabolism.

Summary

The effects of base supply (Ca, K, Mg) and nitrogen source (nitrate *versus* ammonium) on total organic acid and oxalic acid production were investigated with seedling tung trees grown in a nutritional experiment set up in factorial design.

Oxalic acid formation was found to be directly related to nitrate concentration and nitrogen uptake from all nitrate solutions as well as to Ca supply and uptake. Both K and Mg influenced oxalic acid formation by their repressing effect on Ca uptake.

It is suggested that oxalic acid formation in the tung plant is a by-product of the process of oxidation of carbohydrates which furnish energy for reduction of nitrate to protein N. With an all-nitrate supply, a high Ca requirement results from the precipitation of oxalate with the oxalic acid formed in this type of N metabolism. When nitrate reduction is lessened, a correspondingly lower Ca requirement exists for normal growth.

The authors wish to acknowledge the close personal interest and suggestions of Dr. H. L. Crane during the course of this work.

BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING
AGRICULTURAL RESEARCH ADMINISTRATION, U.S.D.A.
GAINESVILLE, FLORIDA

LITERATURE CITED

1. ERGLE, D. R. and EATON, F. M. The organic acids of the cotton plant. Plant Physiol. **24**: 373–388. 1949.
2. GILBERT, S. G. and SHIVE, J. W. The importance of oxygen in the nutrient substrate for plants—relation of the nitrate ion to respiration. Soil Sci. **59**: 453–460. 1945.
3. MILLER, E. C. Plant Physiology. First ed. McGraw-Hill Book Co., New York. 1931.
4. OLSEN, CARSTEN. Absorption of calcium and formation of oxalic acid in higher green plants. Comptes Rendus d. Lab. Carlsb. Ser. Chim. **23**: 101–124. 1939.
5. PEPKOWITZ, L. P., GILBERT, S. G., and SHIVE, J. W. The importance of oxygen in the nutrient substrate for plants—organic acid. Soil Sci. **58**: 295–303. 1944.

6. Pierce, Elwood C. and Appleman, C. O. Role of ether-soluble organic acids in the cation–anion balance in plants. Plant Physiol. **18**: 224–238. 1943.
7. Pucher, G. W., Vickery, H. B., and Wakeman, A. J. Determination of the acids and of plant tissue: II. Total organic acids of tobacco leaf. Ind. and Eng. Chem., Anal. Ed. **6**: 104–143. 1934.
8. Pucher, G. W., Wakeman, A. J., and Vickery, H. B. Organic acids in plant tissues. Modifications of analytical methods. Ind. and Eng. Chem., Anal. Ed. **13**: 244–246. 1941.
9. Shear, C. B., Crane, H. L., and Myers, A. T. Nutrient element balance Potassium, magnesium and calcium interrelations, effects on growth, mineral composition, and minor element nutrition of tung trees in sand cultures. U.S.D.A. Tech. Bul. (in preparation).
10. Shear, C. B., Crane, H. L., and Specht, A. W. Some effects of ammonia–nitrate ratios on cation accumulation in tung leaves. (In preparation.)
11. Snedecor, G. W. Statistical Methods. 4th ed. Iowa State College Press, Ames, Iowa. 1946.
12. Ulrich, Albert. Metabolism of non-volatile organic acids in excised barley roots as related to cation–anion balance during salt accumulation. Amer. Jour. Bot. **28**: 526–537. 1941.
13. Vickery, H. B. and Pucher, G. W. Organic acids of plants. Ann. Rev. Biochem. **9**: 529–544. 1940.
14. Wadleigh, C. H. and Shive, J. W. Organic acid content of corn plants as influenced by pH of substrate and form of nitrogen supplied. Amer. Jour. Bot. **26**: 244–248. 1939.

INVESTIGATIONS OF ASCORBIC ACID DEHYDROGENASE OF PEAS (*PISUM SATIVUM*) AND ITS DISTRIBUTION IN THE DEVELOPING PLANT

M. YAMAGUCHI AND M. A. JOSLYN

(WITH EIGHT FIGURES)

Received December 18, 1950

Introduction

Ascorbic acid has long been considered as a possible hydrogen donor in the intermediary oxidation-reduction chain in living tissues. The biological oxidation of ascorbic acid to dehydro-ascorbic acid by ascorbic acid oxidase, polyphenolase, peroxidase, and other systems has been carefully worked out by many investigators (**24**). In plants, however, ascorbic acid is nearly always found in the reduced state and very little is found as dehydro-ascorbic acid, the oxidized state. Therefore there must be present in the living plant cells some reducing mechanism to maintain the ascorbic acid in the reduced state in spite of the terminal oxidases and oxidizing catalysts which tend to oxidize it to dehydro-ascorbic acid.

Recently several investigators (**5, 9, 15, 16**) have reported that such an enzyme exists in peas. The enzyme, ascorbic acid dehydrogenase, also called dehydro-ascorbic acid reductase and ascorbic reductase, catalyzes the reduction of dehydro-ascorbic acid as given in the following equation:

$$\text{dehydro-L-ascorbic acid} + 2\ \text{glutathione} \xrightarrow[\text{dehydrogenase}]{\text{ascorbic acid}} \text{L-ascorbic acid} + \text{oxidized glutathione.}$$

Review of literature

The first observations of the ability of glutathione to donate hydrogen to dehydro-ascorbic acid, then known as oxidized hexuronic acid, were made by SZENT-GYÖRGYI (**27**) while studying the properties of hexuronic acid (ascorbic acid) isolated from the adrenal cortex. SZENT-GYÖRGYI (**28**) observed that macerated cabbage tissue slightly reduced the oxidized hexuronic acid and that glutathione was oxidized. PFANKUCK (**21, 22**) reported that 0.3% cysteine has the ability to reduce dehydro-ascorbic acid in the presence of potato press juice. This reductive power was thought to be enzymatic because heat destroyed the catalyst and also because protein-free filtrates and pure dehydro-ascorbic acid and cysteine solutions did not cause the reduction.

HOPKINS and MORGAN (**11**) found that ascorbic acid was protected from oxidation by the presence of glutathione in cabbage juice. This protective effect was ascribed to an initial reversible oxidation of ascorbic acid by the

juice, followed by the anaerobic reduction of dehydro-ascorbic acid upon the addition of glutathione to the system. The rate of reduction was five times as rapid as that of oxidation. BORSOOK *et al.* (**3**) showed that dehydro-ascorbic acid added to minced animal tissues was rapidly reduced. They performed experiments to show that reduced glutathione was the principal reducing agent.

KOHMAN and SANBORN (**16**) found that when freshly pressed pea juice was exposed to air during the pressing operation, its ascorbic acid content was lower initially than when the determination was made some time later. They also observed a comparable rise in the reducing value of pea juice after the addition of oxidized ascorbic acid. Reduction of dehydro-ascorbic acid was not inhibited completely by heating the pressed pea juice. They proposed that glutathione might act as an intermediary in the reduction of dehydro-ascorbic acid and concluded that the reduction was enzymatic in nature.

KERTESZ (**14**) was unable to reproduce the experimental results of HOPKINS and MORGAN (**11**) with cauliflower and cucumber extracts. CROOK and MORGAN (**7**) found that in cucumber juice there was but little protection of ascorbic acid by glutathione at pH values near neutrality. However, they found greater protection by glutathione than that reported by KERTESZ (**14**). They accounted for Kertesz's failure to observe the inhibition of ascorbic acid oxidation by the instability of the reducing enzyme system. They believed that there exists in plant tissues a reducing enzyme different from the associated oxidases.

CROOK (**8**) partially purified the reducing enzyme from cauliflower juice and determined some of its properties. He showed that the reducing enzyme and ascorbic acid oxidase were not the same but were two distinctly different enzymes.

BUKIN (**5**) working with the juice from leaves of white cabbage also found that the reducing enzyme was not the same as the ascorbic acid oxidase. Each had a different pH optimum. The reducing enzyme was inhibited by iodoacetic acid and not by cyanide but the opposite was true for the oxidase. He found that dihydro-cozymase reacted non-enzymatically with oxidized glutathione and indirectly influenced the reaction rate by supplying reduced glutathione to the system.

Further work by CROOK and MORGAN (**9**) on the reduction of dehydro-ascorbic acid by plant extracts indicated that the reducing enzyme is not widespread in the plant kingdom. They found that the enzyme was absent in eight species of plants. Members of the Cruciferae and Leguminosae had the highest reducing enzyme activity.

The non-enzymatic reaction between dehydro-ascorbic acid and glutathione has been extensively studied by BORSOOK *et al.* (**3**). Ascorbic acid which had been oxidized by iodine was found to be easily reduced by high concentrations of glutathione *in vacuo* at neutrality. The pH of the solution greatly influenced this reaction; at pH 4.0, less than 1% was reduced;

at pH 7.0, 85% was reduced; and at pH of 8.0, 91% was reduced. They found that, even at high concentrations, glutathione was unable to reduce 2,3-diketo-gulonic acid, a degradation compound of dehydro-ascorbic acid, at neutrality and at 37.5° C.

BUKIN (**5**) found that at pH 6.6 or lower, no reduction of dehydro-ascorbic acid by glutathione took place at .002 *M* concentrations of glutathione and dehydro-ascorbic acid.

Dry pea seeds have very little ascorbic acid or dehydro-ascorbic acid (**6**) but when germinated, ascorbic acid appeared within a fairly short time. ROBERTSON (**26**) conducted experiments on the accumulation of ascorbic acid and dehydro-ascorbic acid in peas from which the embryo had been removed. He found that upon soaking, the peas with the embryo removed produced higher concentration of ascorbic acid than intact germinating pea seeds.

HOPKINS and MORGAN (**12**) found that glutathione was absent in dry pea seeds but appeared rapidly when placed under water. They isolated glutathione from germinating pea seeds.

BUKIN (**5**) found that the ascorbic acid dehydrogenase decreased during the germination of pea seeds. No further work has been reported on the distribution of this enzyme in pea plants.

Experimental procedures

REAGENTS

Glutathione, L-ascorbic acid and 2,6-dichlorophenol indophenol were Eastman Kodak Company's highest purity grade. Titration with standard I_3^- solution showed that the glutathione was completely in the reduced form. All inorganic chemicals were either C.P. or reagent grades. All solutions were prepared with water distilled from all-glass apparatus to avoid cupric ion contamination. This precaution was taken to minimize the cupric ion catalyzed oxidation of ascorbic acid (**2, 18**).

Crystalline dehydro-L-ascorbic acid was prepared according to the method of KENYON and MUNRO (**13**). Solution of the crystals of dehydro-ascorbic acid and subsequent reduction with H_2S gave 85% recovery.

EXTRACTION OF DEHYDROGENASE FROM PEAS

Laxton's Progress peas grown at Davis were used as the source of enzyme. The peas were shelled and frozen immediately after harvesting. They were then mashed in a mortar and the juice pressed through two layers of muslin cloth in a laboratory hydraulic press at 5,000 pounds per square inch pressure. This juice gave a dehydrogenase preparation equal in activity to that of tissue ground at 0° C in a Potter-Elvehjem homogenizer (**25**).

The pressed juice was then centrifuged at 0° C and filtered through a Buchner funnel. Addition of Celite filter aid hastened the filtration. The filtrate was put into small bottles, frozen, and stored at – 10° C. The crude

preparation in this condition retained its activity very well and was used to study some of the properties of the dehydrogenase.

In the study of the distribution of the enzyme, the plants were harvested in the morning and analyzed immediately. They were washed in distilled water and separated into the following parts: roots, cotyledons, stems and pedicle, petioles and tendrils, stipules and leaflets, vegetative buds, and floral buds and flowers. The seeds were soaked over night before they were dissected to separate the embryo from the cotyledons.

The tissues were frozen and a 2.0-gram sample was macerated in a mortar with a few ml. of 0.1 *M* phosphate buffer, pH 6.3. The contents of the mortar were quantitatively transferred to a beaker by washing with the buffer solution, adjusted to pH 6.3 with 0.1 *M* Na_2HPO_4 if necessary, and the volume made to 16 ml. with buffer. The extract was then centrifuged at 2000 r.p.m. in a room at 0° C for 15 minutes. The supernatant liquid was used for the activity measurements. A Beckman Model M meter was used for all pH determinations.

Measurement of activity

The activities of the dehydrogenases were measured in Thunberg tubes made according to Tam and Wilson (**29**). Either 1.0 ml. of the juice and 7.0 ml. of 0.1 *M* phosphate buffer pH 6.3, or 8.0 ml. of tissue extract at pH 6.3 (equivalent to 1 gram of tissue) were pipetted into Thunberg tubes. The side arm contained 2.0 mg. of dehydro-ascorbic acid in 1.0 ml. of 0.1% acetic acid. Seven milligrams of glutathione dissolved in 1.0 ml. of water were pipetted into special glass tubes, small enough to slide down the side arm. This tube was about 2 mm. in diameter and about 25 mm. in length. Lanolin was placed on the rim of the test tube so that the solutions did not mix when the tube was inserted and the solution did not splash out during the evacuation.

The final concentration of the dehydro-ascorbic acid was 1.14×10^{-3} *M* and that of glutathione was 2.28×10^{-3} *M* solution. The volume was 10 ml. The Thunberg tubes were evacuated by means of an oil pump. During the evacuation the solutions tended to foam. This foaming was controlled by means of a 2-way stopcock which turned off the vacuum and let nitrogen gas into the tubes at low pressure (3 lb. per sq. in.). When the tubes were filled, as indicated by a manometer, the tubes were evacuated again. This procedure was repeated twice to remove the dissolved oxygen. After the final evacuation the side arms of the tubes were turned to retain the vacuum. The tubes were placed in a constant temperature bath at 25° C for 15 minutes. The reaction was started by mixing the contents of the tube and allowed to react at 25° C for 10 minutes. The enzyme action was stopped by quickly adding 40 ml. of 1% oxalic acid to the reaction mixture. In the enzyme distribution experiments of pea organs, 1% metaphosphoric acid solution was used since metaphosphoric acid completely precipitated the proteins and the chlorophylls producing a clear filtrate. Several workers

(**4, 10, 18, 23**) have found that metaphosphoric acid was effective in stabilizing ascorbic acid and dehydro-ascorbic acid solutions.

The resultant mixture was at pH 1.5, sufficient to arrest both the enzymatic and non-enzymatic reactions. Control reactions, one without enzyme but with substrate and another with the enzyme but with no substrate, were made to ascertain the non-enzymatic reduction of dehydro-ascorbic acid and also the ascorbic acid content of the enzyme extract for each series of experiments. Usually these control determinations were zero or nearly zero.

Ascorbic acid was estimated by the method of LOEFFLER and PONTING (**17**) using 2,6-dichlorophenol indophenol reagent in an Evelyn photoelectric colorimeter using a 515 mμ filter. The quantity in mg. of ascorbic acid produced in 10 minutes at 25.0° C by 1 gram of tissue or 1 ml. of juice was taken as a measure of dehydrogenase activity. Percentage dry weights of the tissues were determined by the A.O.A.C. methods (**1**). Proteins were determined by the micro-Kjeldahl method. Percentage protein was calculated by multiplying the per cent. nitrogen by the factor, 6.25.

Results and discussion

NON-ENZYMATIC REDUCTION OF DEHYDRO-ASCORBIC ACID BY GLUTATHIONE

The non-enzymatic reduction of dehydro-ascorbic acid by glutathione in the concentrations used in enzymatic activity determinations at various pH's is shown in figure 1. At pH 6.4 and lower, no reduction of dehydro-ascorbic acid was detected, at higher pH's the reduction increased very rapidly. The reduction at pH 7.8 was almost twice that at pH 7.2. BUKIN (**5**) reported that at pH 6.65 no dehydro-ascorbic acid was reduced in 60 minutes but at pH 6.73, 10% was reduced. He used a 0.002 molar concentration of glutathione.

PROPERTIES OF THE DEHYDROGENASE OF PRESSED PEA JUICE

STABILITY OF ENZYME IN PEA JUICE.—Pea juice, with 0.1% sodium ethyl mercuric thiosalicylate added as preservative, was stored at 2° C and 20° C at pH's 6.0 and 7.5 for 10 days. It was most stable at the lower temperature and at the higher pH's. Frozen filtered pea juice kept at – 10° C retained its activity fairly well. Even after 20 weeks the activity dropped to just half of its original value.

RATE OF REDUCTION.—The rate of reduction of dehydro-ascorbic acid to ascorbic acid by pressed pea juice is shown in figure 2. During the first 10 minutes the quantity of dehydro-ascorbic acid reduced appeared to be nearly proportional to time, but for the longer lengths of time the rate fell off rapidly. The same data did not give straight lines when plotted for first and second order reactions. For the first 10 minutes a zero order equation seemed to best fit the data. CROOK (**8**) states that this reaction appears to be complex since dehydro-ascorbic acid undergoes hydrolysis to 2,3-diketogulonic acid at the higher pH value. BORSOOK *et al.* (**3**) found that after

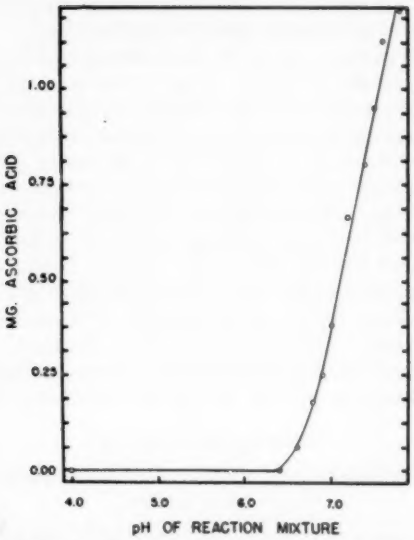


FIG. 1. Non-enzymatic reduction of dehydro-L-ascorbic acid by glutathione at various pH's. Initial concentrations: dehydro-ascorbic acid—2.0 mg. per 10 ml.; glutathione—7.0 mg. per 10 ml. Reaction time—10 minutes. Temperature—25.0° C.

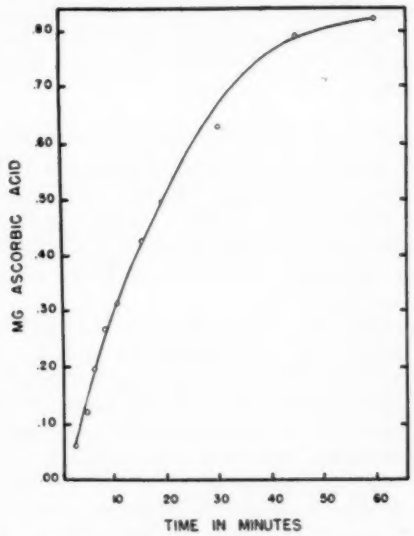


FIG. 2. Rate of reduction of dehydro-L-ascorbic acid to ascorbic acid by the dehydrogenase of pressed pea juice. pH = 6.3. T = 25° C.

five minutes at pH 7.0 and 37° C, 30% of the dehydro-ascorbic acid had hydrolyzed to 2,3-diketo-gulonic acid. PENNY and ZILVA (**19, 20**) reported similar results.

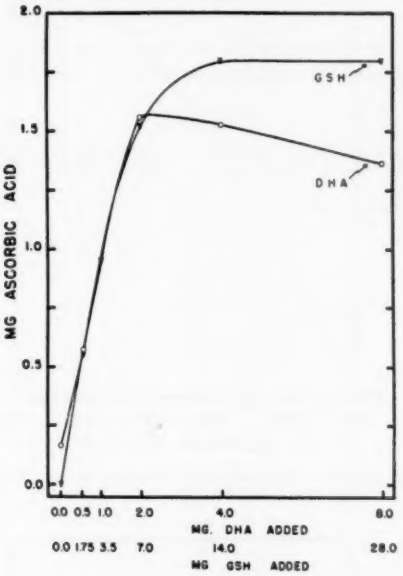


FIG. 3. Effect of substrate concentration on the amount of ascorbic acid produced by the ascorbic acid dehydrogenase. DHA = dehydro-L-ascorbic acid; GSH = glutathione. T = 25° C, pH = 6.3. In the GSH curve the dehydro-ascorbic acid was held constant and the GSH was varied. In the DHA curve the glutathione was held constant and the DHA varied.

EFFECTS OF SUBSTRATE CONCENTRATION.—The effects of the concentrations of dehydro-ascorbic acid and glutathione were studied by keeping one of them constant and varying the concentration of the other. The results are shown in figure 3. The initial value showing activity of about 0.17 when no dehydro-ascorbic acid was added may be due to the dehydro-ascorbic acid already present in the pea juice. The dehydro-ascorbic acid

TABLE I

EFFECT OF BUFFERS ON DEHYDROGENASE ACTIVITY OF PRESSED PEA JUICE

0.1 *M* Buffer (pH 6.3)	Enzyme activity
Orthophosphate	1.38
Metaphosphate	0.81
Citrate	0.83
Acetate	0.80

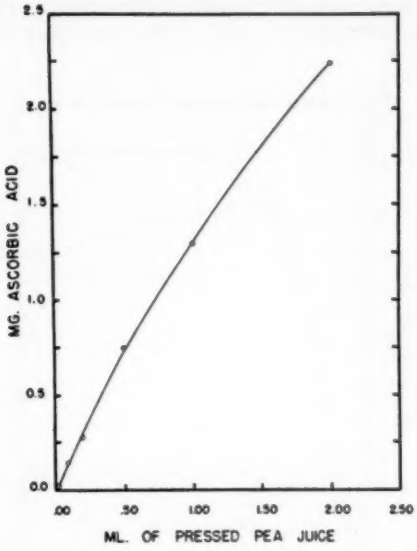


FIG. 4. Effect of amount of pressed pea juice on the quantity of ascorbic acid produced. pH = 6.3. T = 25° C.

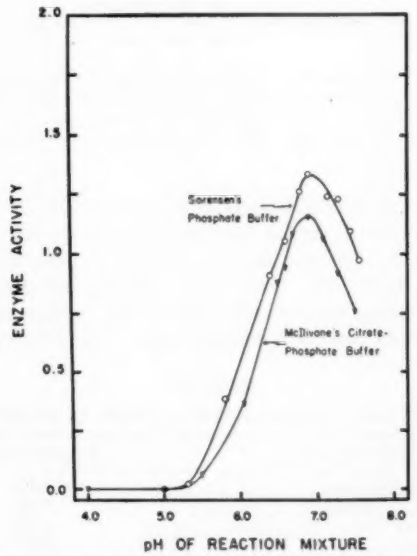


FIG. 5. Enzyme activity of pressed pea juice at various pH's in Sörensen's phosphate buffer and in McIllivane's citrate-phosphate buffer. T = 25° C.

content of pressed pea juice was about 0.3 mg. per ml. of juice. Under the conditions of the experiment, varying the concentration over 2.0 mg. of dehydro-ascorbic acid and over 7.0 mg. of glutathione had little effect on the activity. These concentrations were used in all subsequent experiments unless otherwise noted.

EFFECT OF ENZYME CONCENTRATION.—The quantity of dehydro-ascorbic acid reduced appears to be directly proportional to the enzyme content for

TABLE II

ASCORBIC ACID DEHYDROGENASE CONTENT OF PEA PLANT ORGANS

Age of plant, days	Enzyme activity*		
	Fresh basis	Dry basis	Protein basis
1. Cotyledon			
0	.301	0.92	2.8
2.5	.920	2.85	9.2
5	.930	3.07	8.0
14	.065	0.65	2.3
28	.016	0.11	1.3
2. Embryo			
0	4.93	25.3	48.7
2.5	1.02	11.1	28.1
3. Root			
5	.295	4.21	14.1
14	.039	1.04	3.2
28	.054	1.53	6.3
49	.098	1.64	11.0
77	.115	1.20	8.1
4. Stem and pedicle			
5	.273	3.78	10.2
14	.273	4.65	13.7
28	.187	3.50	15.3
49	.060	0.65	3.7
77	.273	1.86	17.2
5. Petiole and tendril			
28	.164	2.73	8.3
49	.060	0.65	2.8
77	.109	0.88	6.9
6. Leaflet and stipule			
14	.765	6.35	11.7
28	.655	6.65	13.4
49	.910	7.90	20.3
77	.535	4.70	16.8
7. Bud (vegetative)			
5	1.57	15.7	31.3
14	2.62	20.2	33.9
28	2.66	20.4	37.3
49	2.75	15.3	35.0
8 Bud (floral) and flowers			
49	2.71	16.9	45.4
77	0.85	5.2	12.1

*Enzyme activity—milligrams of ascorbic acid produced in 10 minutes at 25° C by 1 gram of tissue.

values up to 0.5 ml. of juice, as shown in figure 4. CROOK (**8**) found that the amount of ascorbic acid formed was proportional to the enzyme concentration for as much as 3 ml. of cabbage or broccoli juice.

EFFECT OF pH ON ACTIVITY.—The effect of pH on the dehydrogenase was measured in Sörensen's phosphate buffer and in McIllivane's citrate phosphate buffer. Figure 5 shows the net activities of the enzyme in the two buffer solutions. The dehydrogenase seemed to be very sensitive to acid and at pH 5.0 or lower, the activity disappeared entirely. Above pH 7.5 the non-enzymatic reduction of dehydro-ascorbic acid took place very rapidly and it was very difficult to obtain accurate activity measurements. The

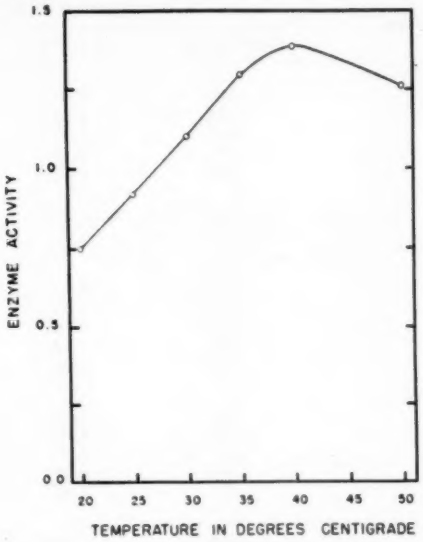


FIG. 6. Effect of temperature on dehydrogenase activity of pressed pea juice. pH = 6.3.

non-enzymatic reduction was determined simultaneously in these tests and corrected for in the graphs. The optimum pH appears to be about 6.9 for both buffers. No comparison can be made between the two buffers because different samples were used. CROOK and MORGAN (**7**), CROOK (**8**), and BUKIN (**5**) reported an optimum pH 6.7 to 6.8.

EFFECT OF TYPES OF BUFFERS.—Solutions of orthophosphate, metaphosphate, citrate, and acetate at pH 6.3 were used to study the effect of buffers on the dehydrogenase activity of the pea juice. The orthophosphate solution was made with 0.1 *M* KH_2PO_4 and Na_2HPO_4. Metaphosphate, citrate and acetate solutions were made by neutralizing 0.2 *M* solutions of the cor-

responding acids with concentrated NaOH solution and diluting them to 0.1 *M* concentration. Table I shows the effect of these buffers on the enzyme activity. The results indicate that the dehydrogenase was most active in the orthophosphate and the activities in the metaphosphate, citrate, and acetate were approximately the same.

EFFECT OF TEMPERATURE ON RATE AND STABILITY.—Figure 6 shows the effect of temperature on the activity of the enzyme. The reaction vessels were set in a water bath and at various temperatures for 10 minutes before allowing the reaction to take place. The maximum reduction occurred at

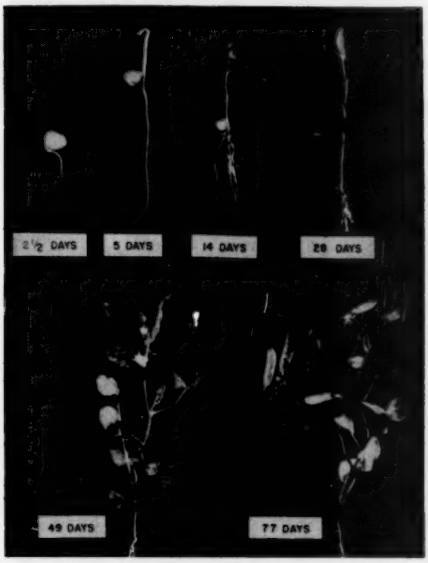


FIG. 7. Photographs of growing pea plants showing the several stages of maturity at which they were analyzed for ascorbic acid dehydrogenase activity. Period is days after planting.

40° C. The correction was made for non-enzymatic reduction. The dehydrogenase was very unstable at the higher temperatures, 26% being inactivated in three minutes at 50° C, 94% at 60° C, and 100% at 70° C.

EFFECT OF DIALYSIS.—The enzyme was rapidly inactivated when dialyzed through a cellophane membrane with running distilled water at room temperature. Pea juice dialyzed against large volumes of 0.1 *M* phosphate buffer pH 6.8 and at a temperature of about 1° C retained its activity fairly well. The activity was lost after four days dialysis in buffer or distilled water. The activity was not restored on addition of boiled pea juice.

Distribution of the ascorbic acid dehydrogenase at different stages of maturity of the pea plant

Figures 7 and 8 are photographs of the pea plants and pea seeds used in the determinations. The age of the plants was taken from the date of planting and the age of the seeds was taken from the date of bloom. About five days elapsed before the plumule emerged through the soil. Table II gives the enzyme activity of the different organs of the plant. The highest activity appeared to be in the meristematic region where the respiration rate

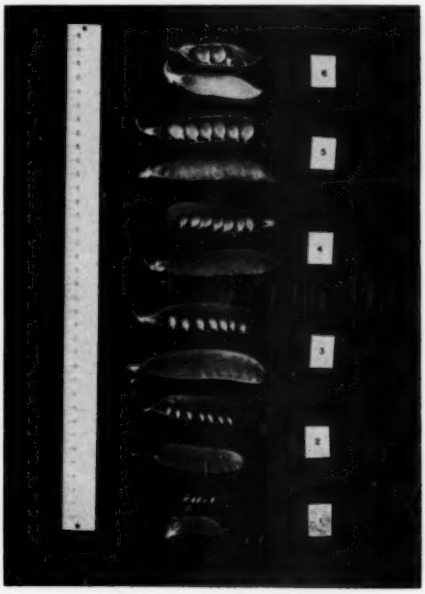


FIG. 8. Appearance of pods and seeds of the pea plant at several stages of growth following blossoming. 1 to 5 days, 2 to 10 days, 3 to 14 days, 4 to 18 days, 5 to 21 days, 6 to 28 days after full bloom.

is very high. Leaflets and stipules were also high in dehydrogenase activity while the petioles and tendrils, stems and roots were low. The cotyledons were high at germination, but after 14 days the activity dropped to nearly zero.

Tables III and IV give the size of the pea seeds and pods and the activity at several maturities. Five days after full bloom the pods were still elongating. The youngest seeds seem to have the highest activity and the dry seeds the least on both dry and protein bases. The activity decreased very rapidly during the 14-day period following blossoming.

TABLE III

EFFECT OF MATURITY ON DEHYDROGENASE ACTIVITY OF PEA SEEDS

Stage of maturity	Age, days after full bloom	Diameter of pea, mm.	Ave. wt. per pea seed gm.	Enzyme activity*		
				Fresh wt. basis	Dry wt. basis	Protein basis
1 (immature)	5	2.3- 3.2	.0095	3.41	22.4	61.2
2	10	3.2- 4.5	.048	2.63	16.7	53.5
3	14	4.9- 6.4	.16	1.78	10.0	37.2
4	18	7.3- 8.8	.33	1.50	7.9	30.3
5 (market mature)	21	9.6-11.2	.62	1.75	7.5	28.3
6	28	11.2-12.8	.80	1.69	5.3	18.9
7 (dry pea seeds)	....	7.3- 8.8	.30	4.11	4.5	16.4

*Enzyme activity—milligrams of ascorbic acid produced in 10 minutes at 25° C by 1 gram of tissue.

The pods were not completely elongated five days after full bloom of the flowers, but elongation was complete after 14 days and the pea seeds commenced to increase in size. The enzyme activity on both the fresh and dry weight basis decreased as the pods matured; however, the activity remained about the same on the protein basis.

By expressing the enzyme activity on the protein basis, instead of the fresh weight basis, an attempt was made to determine whether the dehydrogenase content of the organs was the same throughout the life cycle of the pea plant. No correlation can be made on this basis except that the differences of the enzyme contents of the different tissues were not as great as on the fresh or dry weight basis.

The reversible reaction of ascorbic acid and dehydro-ascorbic acid has long been associated with cellular activity (**6**). Since dehydro-ascorbic acid and glutathione appear in the germinating pea seeds, ascorbic acid dehydrogenase may well play an important role in the developing embryo. The enzyme seems to be concentrated in tissues of high metabolic activity, since the growing point region, leaves and embryo of the pea seed have the highest dehydrogenase activity.

TABLE IV

EFFECT OF MATURITY ON DEHYDROGENASE ACTIVITY OF PEA PODS

Stage of maturity	Age, days after full bloom	Ave. wt. per pod grams	Enzyme activity*		
			Fresh wt. basis	Dry wt. basis	Protein basis
1 (immature)	5	1.4	.175	1.42	4.21
3	14	4.2	.120	0.87	4.32
5 (mature)	21	3.7	.109	0.76	4.37

*Enzyme activity—milligrams of ascorbic acid produced in 10 minutes at 25° C by 1 gram of tissue.

Summary

Properties of the ascorbic acid dehydrogenase present in pressed pea juice and the enzyme content in different organs of the pea plant at several stages of maturity were studied.

The enzyme was soluble in the pressed pea juice. The dehydrogenase in the juice was fairly stable when frozen and stored at – 10° C. It lost less than one-half of its activity in 20 weeks but when stored at a higher temperature, the enzyme was rapidly inactivated.

The dehydrogenase was very thermolabile and was very rapidly inactivated at 50° C. Activity was highest in orthophosphate buffer and at pH 6.9.

In germinating pea seeds, the embryo had the highest dehydrogenase activity and the activity in the cotyledons decreased from a fairly high value at the beginning of germination to just a trace when the pea seedling was able to synthesize its own food supply. The growing point and leaves had high enzyme activity. The youngest pea seeds had the highest concentration. Pods were very low in dehydrogenase content.

University of California
Division of Truck Crops
Davis, California
and
Division of Food Technology
Berkeley, California

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. Ed. 6. Washington, D. C. 1945.
2. Barron, E. S. G., Deio, R. H., and Klemperer, F. Studies on biological oxidation. V. Copper and hemochrome as catalysts for the oxidation of ascorbic acid. The mechanism of the oxidation. Jour. Biol. Chem. **112**: 625–640. 1935.
3. Borsook, H., Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C. The oxidation of ascorbic acid and its reduction *in vitro* and *in vivo*. Jour. Biol. Chem. **117**: 237–279. 1937.
4. Brown, H. and Meyer, E. Experimentelle Untersuchungen über die Stabilisierung von Vitamin C in Wässerigen Lösungen, Hagebuttenauszügen and Urin durch Metaphosphorsäure. Biochem. Zeit. **319**: 300–304. 1949.
5. Bukin, V. N. Enzymatic reduction of dehydro-ascorbic acid (translated from Russian). Biokhimiia **8**: 60–76. 1943.
6. Carroll, G. H. The role of ascorbic acid in plant nutrition. Bot. Rev. **9**: 41–48. 1943.
7. Crook, E. M. and Morgan, E. J. Further observation on the system ascorbic acid glutathione–ascorbic acid–oxidase. Biochem. Jour. **32**: 1356–1363. 1938.

8. Crook, E. M. The system dehydro-ascorbic acid–glutathione. Biochem. Jour. **35**: 226–236. 1941.
9. Crook, E. M. and Morgan, E. J. The reduction of dehydro-ascorbic acid in plant extracts. Biochem. Jour. **38**: 10–15. 1944.
10. Guild, L. P., Lockhart, E. F., and Harris, R. S. Stability of solutions of pure ascorbic acid and of dehydro-ascorbic acid. Science **107**: 226–227. 1947.
11. Hopkins, F. G. and Morgan, E. J. Some relations between ascorbic acid and glutathione. Biochem. Jour. **30**: 1446–1462. 1936.
12. Hopkins, F. G. and Morgan, E. J. Appearance of glutathione during the early stages of germination of seeds. Nature **152**: 288–290. 1943.
13. Kenyon, J. and Munro, N. The isolation and some properties of dehydro-l-ascorbic acid. Jour. Chem. Soc. 158–161. 1948.
14. Kertesz, Z. I. Observations on the system ascorbic acid–glutathione–ascorbic acid oxidase. Biochem. Jour. **32**: 621–625. 1938.
15. Kohman, E. F. and Sanborn, N. H. Dehydro-ascorbic acid reductase. Ind. Eng. Chem. **29**: 189–190. 1937.
16. Kohman, E. F. and Sanborn, N. H. Vegetal reduction of dehydro-ascorbic acid. Ind. Eng. Chem. **29**: 1195–1199. 1937.
17. Loeffler, H. J. and Ponting, J. D. Ascorbic acid—Rapid determination in fresh, frozen, or dehydrated fruits and vegetables. Ind. Eng. Chem., Anal. Ed. **14**: 846–849. 1942.
18. Muslin, R. R. and King, C. G. Metaphosphoric acid in the extraction and titration of vitamin C. Jour. Biol. Chem. **116**: 409–413. 1936.
19. Penny, J. R. and Zilva, S. S. The determination of 2:3-diketo-l-gulonic acid. Biochem. Jour. **37**: 39–44. 1943.
20. Penny, J. R. and Zilva, S. S. The chemical behavior of dehydro-l-ascorbic acid *in vitro* and *in vivo*. Biochem. Jour. **37**: 403–417. 1943.
21. Pfankuck, E. Enzymatische Reduktion von Dehydro-ascorbinsäure. Naturwissenschaften **22**: 821. 1934.
22. Pfankuck, E. Zur Biochemie des Kertoffelabbawes. 3. Ascorbinsäure, Glutathione und Zucker. Biochem. Zeit. **279**: 115–130. 1935.
23. Ponting, J. D. Extraction of ascorbic acid from plant materials. Related suitability of various acids. Ind. Eng. Chem., Anal. Ed. **15**: 389–391. 1943.
24. Ponting, J. D. and Joslyn, M. A. Ascorbic acid oxidation and browning in apple tissue extracts. Arch. Biochem. **19**: 47–63. 1948.
25. Potter, V. R. and Elvehjem, C. A. A modified method for the study of tissue oxidations. Jour. Biol. Chem. **114**: 495–504. 1936.
26. Robertson, T. S. B. The accumulation of vitamin C during germination in peas. Australian Jour. Exptl. Biol. Med. Sci. **25**: 41–46. 1947.

27. SZENT-GYÖRGYI, A. Observation on the function of peroxidose system and the chemistry of the adrenal cortex—Description of a new carbohydrate derivative. Biochem. Jour. **22**: 1387–1409. 1928.

28. SZENT-GYÖRGYI, A. On the function of hexuronic acid in the respiration of the cabbage leaf. Jour. Biol. Chem. **90**: 385–393. 1931.

29. TAM, R. K. and WILSON, P. W. Respiratory enzyme system in symbiotic nitrogen fixation. III. The dehydrogenase systems of *Rhizobium trifolii* and *Rhizobium leguminosarum*. Jour. Bact. **41**: 529–546. 1941.

THE RELATIVE HERBICIDAL EFFECTIVENESS OF SEVERAL DERIVATIVES OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXY-ACETIC ACID

WENDELL R. MULLISON

Received December 26, 1950

Introduction

Several papers have been published by ZIMMERMAN and HITCHCOCK (**10, 11, 12**) in which the activity of various derivatives of plant growth regulating substances has been discussed. AVERY and his associates (**1**) have published results concerning the activity of esters of plant growth regulators as compared with salts and acids as shown by the Avena test. More recently, MURRAY and WHITING (**6**) and HAMNER, LUCAS, and SELL (**4**) have discussed this problem. In the Proceedings of the North Central Weed Control Conference for 1946 and 1947 several investigators have published studies concerning the effect of the acid as compared with certain salts and esters of 2,4-dichlorophenoxyacetic acid (2,4,-D). In 1948 and 1949, the general feeling expressed in the Proceedings and Research Publication of the North Central Weed Control Conference was that the amine salts of 2,4-D were more effective than the sodium or ammonium salts and that the esters were the most effective of all the 2,4-D derivatives. However, there are few reports as to the activity of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its derivatives. It was thought desirable to compare the activity of several 2,4,5-T derivatives as well as those of 2,4-D.

Methods and results

Cranberry beans and tomatoes were used as the test plants. The compounds with the exception of the amine and ester were all made up by dissolving them directly in water. The two exceptions were commercial formulations which were mixed with water to give the desired concentrations. Preliminary work was done in which the various ingredients of the commercial formulations, such as the emulsifying agent, were added to the other compounds in solution. They were found not to affect the relative standings of the compounds within each experimental group.

In each experiment all compounds were made up to the same concentration based on the 2,4-D or 2,4,5-T acid equivalent weight of the compound. The concentrations used varied with the experiment particularly since 2,4,5-T compounds are often not as soluble as those of 2,4-D. The concentration used is given later for each experiment. The formulations were tested using the single drop bean technique (**3, 7, 9**) and the tomato dip technique (**5**). The drops were applied by means of a micrometer driven

TABLE I

WEIGHT IN GRAMS OF 10 BEAN SHOOTS FROM PLANTS TREATED WITH 2,4-D COMPOUNDS

Compound	Replicates				Average
	1	2	3	4	
2,4-D acid	1.83	2.16	2.62	1.28	1.97
Ca salt 2,4-D	6.65	6.86	5.84	7.44	6.69
Na salt 2,4-D	6.02	5.35	6.21	6.28	5.96
NH_4 salt 2,4-D	4.70	3.61	5.24	3.96	4.37
Alkanol amine salt 2,4-D	1.30	1.41	1.25	1.09	1.26
Control	8.35	8.78	7.94	7.30	8.09

syringe. The experiments were all repeated, in some cases numerous times. Results were in general similar to those reported here. Therefore for the sake of brevity, detailed data will be given only for representative trials.

Experiment one

Ten bean plants were used for each treatment and each treatment was replicated four times. Five compounds, shown in the following table, were used. The concentration of each was 1000 p.p.m. of 2,4,-D acid equivalent. A drop, 0.006 ml. in volume, was applied to the midrib of one of the primary leaves about ½ inch from the juncture of the blade and petiole. After ten days that portion of the plant above the primary leaves was harvested and weighed. Results are given in table I. The data indicate a greater effectiveness of the 2,4-D acid and its alkanolamine salt as compared with the other salts used.

Experiment two

This experiment determined the effects of several 2,4,5-T derivatives as shown by the single drop bean test with ten plants per treatment, each treatment being replicated four times. The concentration used was 500 p.p.m. in each case and two drops (0.012 ml.) were applied to each primary leaf. The data of table II indicate the greater effectiveness of the 2,4,5-T acid and its amine salt as compared with the other salts. The much greater effectiveness of the amine salt of 2,4,5-T as compared with 2,4,5-T is also

TABLE II

WEIGHT IN GRAMS OF 10 BEAN SHOOTS FROM PLANTS TREATED WITH 2,4,5-T COMPOUNDS

Compound	Replicates				Average
	1	2	3	4	
2,4,5-T acid	9.44	9.61	7.09	8.72	8.72
Ca salt 2,4,5-T	13.09	12.46	13.50	13.76	13.20
Na salt 2,4,5-T	14.66	12.82	12.32	14.12	13.48
NH_4 salt 2,4,5-T	13.62	14.15	12.86	12.94	13.39
Alkanol amine salt 2,4,5-T	1.69	1.58	1.74	1.72	1.68
Control	12.51	....	....	11.84	12.18

TABLE III

WEIGHT IN GRAMS OF 10 BEAN SHOOTS FROM PLANTS TREATED WITH 2,4-D AND 2,4,5-T COMPOUNDS

Derivative	2,4-D	2,4,5-T
Acid	21.4	22.4
Alkanolamine salt	24.3	7.9
Isopropyl ester	15.9	3.1
Control	26.7	30.4

of interest, particularly when compared with their 2,4-D homologs where such is not the case.

Experiment three

This experiment was designed to make a comparison between the effectiveness of the acid, the alkanolamine salt, and the isopropyl ester of 2,4-D and of 2,4,5-T respectively. In this case six drops, each containing 0.006 ml. of a 250 p.p.m. acid equivalent solution, were applied to each bean plant. These drops were placed on the midribs of the primary leaves about one half inch from the juncture of the blade and the petiole. Otherwise the procedure was the same as in the other experiments. Ten bean plants were used per treatment and the weight is expressed in grams. The data in table III show an interesting case of specificity of these materials; the amine and ester of 2,4,5-T are much more active than the corresponding derivatives of 2,4-D. The data also indicate the herbicidal superiority of ester formulations over other 2,4-D and 2,4,5-T derivatives.

Experiment four

This experiment gives the data obtained when tomatoes were used as the test plants. The free acid, the calcium salt, the sodium salt, the ammonium salt, an alkanolamine salt, and the isopropyl ester of 2,4-D were applied to tomato plants. The same derivatives of 2,4,5-T were also tested. Three concentrations, 25, 250, and 500 p.p.m. acid equivalent, were used. Five tomato plants were treated at each concentration. The tomato dip technique was used. The plants were rated individually on a basis from 0 to 100 (5), then averaged, with zero indicating no effect and 100 indicating that the plants were dead. Results are given in table IV.

TABLE IV

HERBICIDAL EFFECT OF SEVERAL 2,4-D AND 2,4,5-T DERIVATIVES ON TOMATOES

Derivative	2,4-D			2,4,5-T		
	500	250	25	500	250	25
Acid	80	74	44	84	50	0
Ca salt	62	58	15	....	28	0
Na salt	78	72	15	68	34	0
NH_4 salt	88	74	15	82	50	0
Amine salt	84	80	25	84	62	6
Isopropyl ester	100	80	62	100	84	52

The results of this experiment indicate several points: first, on tomatoes there is little difference between the ester formulations of 2,4-D and 2,4,5-T; second, there is more leaf modification with 2,4-D than 2,4,5-T compounds, this being most noticeable at the 25 p.p.m. concentrations; third, the differences between the derivatives of 2,4-D are less marked than they are with the derivatives of 2,4,5-T. This is attributed to the lower solubility of the latter compounds. It is possible that they do not enter the plant and for this reason the ester and amine of 2,4,5-T are much more effective than the other 2,4,5-T derivatives tested here.

Discussion

It is interesting to note that within the group of 2,4-D or 2,4,5-T derivatives the relative order of activity was about the same for both the beans and tomatoes. These data also support the fact first mentioned by Swanson (**7**) that 2,4,5-T is more active on beans than 2,4-D. The 2,4,5-T compounds are often more active on members of the Leguminosae; Tam (**8**) made use of this in his studies on herbicides in Hawaii. Almost simultaneously, Barrons and Coulter (**2**) noted the greater effectiveness of 2,4,5-T on members of the genus Rubus, particularly blackberries. These facts have been very useful in practical weed control work.

It is also of interest to note that the data for tomatoes do not show any great superiority of either 2,4-D or 2,4,5-T over the other. However, 2,4-D is superior in that formative effects such as leaf modifications are brought about at lower concentrations by it than by 2,4,5-T. It is suggested from the results of the experiments presented here that the effectiveness of certain derivatives of 2,4-D and 2,4,5-T is due to more rapid entry into the plants rather than to any greater herbicidal effectiveness per se. It is hard to conceive of any difference in the effect of ionizable 2,4-D, *e.g.* alkali metal or ammonium salts, once the material is in the plant, since the 2,4-D would exist as anions in the sap. The esters and the free acid are more oil soluble than are the salts. This property would be expected to facilitate passage of the former through the waxy cuticle of plants. The same line of reasoning is also supported by the fact that the more insoluble salts, especially those of 2,4,5-T, are less active.

Conclusions

The herbicidal activities of the 2,4-D derivatives used in these tests may be ranked as follows: isopropyl ester > alkanolamine salt > 2,4-D acid > ammonium salt > sodium salt > calcium salt.

The herbicidal activities of the 2,4,5-T derivatives used in these tests may be ranked as follows: isopropyl ester > alkanolamine salt > 2,4,5-T acid > ammonium, sodium and calcium salts.

Biochemical Research Department
Dow Chemical Company
Midland, Michigan

LITERATURE CITED

1. AVERY, G. S. JR., BURKHOLDER, P. R., and CREIGHTON, H. B. Avena coleoptile curvature in relation to different concentrations of certain synthetic substances. Amer. Jour. Bot. **24**: 226–232. 1937.
2. BARRONS, K. C. and COULTER, L. L. The specific effect of 2,4,5-trichlorophenoxy acetic acid on members of the genus Rubus and certain other 2,4-D resistant species. Proceedings 4th Annual Meeting of the North Central Weed Control Conference and Report of Research Committee. p. 255. 1947.
3. BROWN, J. W. and WEINTRAUB, R. L. A leaf repression method for evaluation of formative activity of plant growth-regulating chemicals. Bot. Gaz. **111**: 448–456. 1950.
4. HAMNER, C. L., LUCAS, E. H., and SELL, H. M. The effect of different acidity levels on the herbicidal action of the sodium salt of 2,4-dichlorophenoxyacetic acid. Michigan Agr. Exp. Sta. Quart. Bull. **29**: 337–342. 1947.
5. MULLISON, W. R. The tomato as a test plant for screening and evaluating plant growth regulators. Bot. Gaz. **112**: 521–524. 1951.
6. MURRAY, M. A. and WHITING, A. G. A comparison of the effectiveness of 2,4-dichlorophenoxy acetic acid and four of its salts in inducing histological responses in bean plants. Bot. Gaz. **109**: 13–39. 1947.
7. SWANSON, C. P. Two methods for the determination of the herbicidal effectiveness of plant growth regulating substances in oil solution on broadleaf plants. Bot. Gaz. **107**: 560–562. 1946.
8. TAM, R. K. Comparative herbicidal value of 2,4-dichlorophenoxyacetic acid on some herbaceous weeds, shrubs, and trees under Hawaiian conditions. Bot. Gaz. **109**: 194–203. 1947.
9. THOMPSON, H. E., SWANSON, C. P., and NORMAN, A. G. New growth-regulating compounds. I. A summary of growth-inhibitory activities of some organic compounds as determined by three tests. Bot. Gaz. **107**: 476–507. 1946.
10. ZIMMERMAN, P. W., HITCHCOCK, A. E., and WILCOXON, F. Several esters as plant hormones. Contr. Boyce Thompson Inst. **8**: 105–112. 1936.
11. ZIMMERMAN, P. W. and HITCHCOCK, A. E. Comparative effectiveness of acids, esters, and salts as growth substances and methods of evaluating them. Contr. Boyce Thompson Inst. **8**: 337–350. 1937.
12. ZIMMERMAN, P. W. and HITCHCOCK, A. E. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contr. Boyce Thompson Inst. **12**: 321–343. 1942.

THE GROWTH CAPACITY OF THE SUNFLOWER HYPOCOTYL

R. S. DE ROPP[1]

(WITH TWO FIGURES)

Received February 26, 1951

Since the early studies of SACHS (**2**) on the elongation of roots and stems, it has been realized that the capacity for growth of these plant organs is not equal along the whole of their lengths but varies from one region to another. The present study was designed to give information as to the growth capacities of excised fragments taken from the different regions of sunflower hypocotyl. The growth of these fragments was studied under three sets of conditions: (1) on sucrose mineral agar, (2) on sucrose mineral agar to which indole-3-acetic acid (IAA) had been added, (3) on sucrose mineral agar after inoculation of the fragment with crown-gall bacteria. Five aspects of growth were measured: increase in length, breadth, fresh and dry weight, and production of adventitious roots.

Sunflower seeds var. Russian Giant were sterilized by being immersed for two minutes in a 1% solution of sodium hypochlorite. They were then washed in three changes of sterile distilled water and soaked in water for two hours. The embryos were then removed from their seed coats, transferred aseptically to tubes (25 mm. × 200 mm.) each containing 10 ml. nutrient agar (2% sucrose + White's mineral solution + 1% agar) and cultured at 25° C in continuous light from four 40 watt fluorescent lamps. When the hypocotyls attained a length of 100 mm. the embryos were drawn out of their tubes and the hypocotyls severed just above the root and immediately below the cotyledons. The hypocotyls were cut into segments five mm. long which were placed radicle end uppermost in agglutination tubes (10 mm. × 75 mm.) containing three ml. of nutrient agar. They were incubated for 10 days in darkness at 25° C, then removed, photographed, and weighed. The photographs were made by projecting the image of the fragment through an enlarger onto printing paper. The magnification used was 1.7 diameters in most instances. The length and breadth of the fragment and the size of the tumors were estimated from these shadowgraphs. *Agrobacterium tumefaciens*, strain B.P., was used in these experiments, suspensions being prepared from 24 hour slopes. Indoleacetic acid was incorporated into the nutrient agar for the hypocotyl fragments after autoclaving.

Growth of hypocotyl fragments on sucrose mineral agar

Ten sunflower hypocotyls, each about 100 mm. long, were divided into 20 segments five mm. long; and every alternate fragment beginning with

[1] Present address: Lederle Laboratories Division, Amer. Cyanamid Co., Pearl River, New York.

the one nearest the cotyledons was placed in a tube of sucrose mineral agar. The distances of the foliar ends of these fragments from the points of attachment to the cotyledons were thus: 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 mm. After ten days of incubation in continuous darkness at 25° C the fragments were removed, photographed; and the fresh and dry weights were determined. The mean length, breadth, fresh and dry weight of the fragment, the number of fragments with adventitious roots, and mean number of roots per fragment were measured (table I, fig. 1).

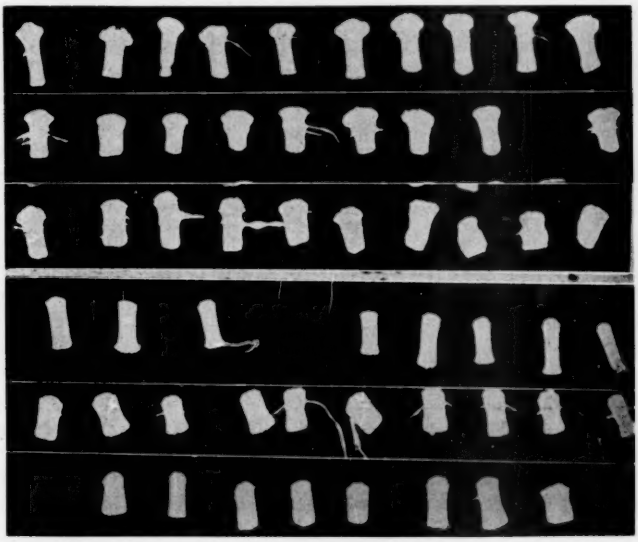


FIG. 1. Upper three rows: fragments of sunflower hypocotyl excised 0 mm. (top row), 40 mm. (middle row) and 90 mm. (bottom row) from point of attachment of cotyledons. Inoculated with crown-gall bacteria, cultured 10 days on nutrient agar. Lower three rows: uninoculated fragments from the same three regions.

Growth in length was greatest in fragments taken from the apex of the hypocotyl, though the fragments in the group nearest the base also elongated significantly more than did those from the center. Increase in width was least in the apical fragments but was about equal in all fragments from 20 mm. down. Production of adventitious roots did not occur at all on the fragments of the apical group but increased in frequency through the 10, 20, and 30 mm. groups, attaining a maximum value in the 40 mm. and lower groups. Numbers of roots per fragment did not differ significantly amongst groups below 10 mm. Both mean fresh and dry weights were significantly higher in groups below the 10 mm. level.

TABLE I

GROWTH ON SUCROSE MINERAL AGAR OF FIVE MM. FRAGMENTS FROM DIFFERENT REGIONS OF THE SUNFLOWER HYPOCOTYL (MEANS OF 10 ESTIMATES)

Distance from cotyledons	Length	Breadth	Weight		Fragments with roots	Roots per fragment
			Fresh	Dry		
mm.	*mm.*	*mm.*	*mg.*	*mg.*		
0	7.0	2.3	29.5	2.3	0	0
10	6.2	2.5	32.1	2.3	3	2
20	5.8	2.8	34.1	2.5	3	3
30	5.0	2.7	36.0	2.8	5	3
40	5.5	2.8	39.3	2.8	7	2
50	5.8	2.8	37.8	3.0	8	2
60	5.6	2.8	37.6	3.0	7	2
70	5.2	3.0	41.0	3.1	8	2
80	5.8	3.0	44.0	3.1	8	2
90	6.4	3.0	31.3	3.0	8	2

Growth of fragments inoculated with crown-gall bacteria

This experiment was similar to the previous one; but the hypocotyl fragments, after being placed on nutrient agar, were inoculated on the freshly cut upper surface with about 1×10^7 viable organisms of *Agrobacterium tumefaciens* strain B.P. These fragments were also weighed and photographed at the end of 10 days incubation in continuous darkness (table II, fig. 1).

Many changes in the growth pattern of these fragments were induced by inoculation with crown-gall bacteria. The increase in length reached a maximum in the two apical groups and was significantly greater than was the increase in the corresponding uninoculated series. The maximum breadth was considerably increased owing to tumor formation on the inoculated end of the fragment. The breadth of the tumors was about the same

TABLE II

GROWTH OF FRAGMENTS OF SUNFLOWER HYPOCOTYL INOCULATED WITH CROWN-GALL BACTERIA (MEANS OF 10 ESTIMATES)

Distance from apex	Length	Breadth	Weight		Fragments with roots	Roots per fragment
			Fresh	Dry		
mm.	*mm.*	*mm.*	*mg.*	*mg.*		
0	7.7	4.3	43.7	2.9	7	2
10	6.9	4.2	41.4	2.9	7	3
20	6.4	4.0	38.2	2.3	7	4
30	6.0	4.0	38.6	2.5	6	4
40	6.0	4.2	40.0	2.8	5	5
50	6.1	4.0	41.5	3.1	3	6
60	5.7	4.1	41.1	3.2	6	4
70	5.9	3.6	36.3	2.9	4	4
80	6.1	4.0	41.8	2.9	4	4
90	6.2	3.7	41.8	2.5	7	3

in all 10 groups. The frequency of occurrence of adventitious roots was greatest in the apical segments, and the mean number of adventitious roots was greatest in the central segments. Both frequency and mean number of roots per fragment were significantly higher in the fragments of the inoculated group as compared with those of the uninoculated group. The fresh and dry weights of the apical fragments in the inoculated group were significantly higher than those of the corresponding uninoculated group.

Growth of fragments in the presence of IAA

To determine the effect of the position of the fragment on its reaction to IAA, 40 sunflower hypocotyls were divided into fragments 5 mm. long. Only the fragments at 0, 30, 60, and 95 mm. from the apex were used. Forty fragments from each level were divided into four groups of 10 fragments each. Fragments in group 1 were placed on agar containing 1 mg./l. IAA, those in group 2 on 0.1 mg./l. IAA, those in group 3 on 0.01 mg./l. IAA, those in group 4 on agar devoid of IAA. The experiment thus contained fragments from four regions of the hypocotyl on four concentrations of IAA, each treatment being replicated 10 times, involving 160 fragments in all.

After 10 days of incubation in continuous darkness at 25° C the fragments were removed, photographed and weighed (table III, fig. 2). In the presence of IAA, the elongation of fragments in the apical group was increased as compared with that of the controls. This effect of IAA on elon-

TABLE III

GROWTH OF FRAGMENTS FROM FOUR REGIONS OF THE SUNFLOWER HYPOCOTYL ON NUTRIENT AGAR CONTAINING IAA

IAA conc.	Distance from apex	Length	Breadth	Weight		Fragments with roots	Roots per fragment
				Fresh	Dry		
mg./l.	*mm.*	*mm.*	*mm.*	*mg.*	*mg.*		
1.0	0	9.7	6.2	100.0	7.7	5	2
	30	6.8	5.2	84.0	6.2	8	4
	60	6.4	6.0	87.0	7.5	5	2
	95	6.4	5.5	107.5	8.2	8	2
0.1	0	9.1	6.0	95.5	6.1	9	3
	30	6.9	5.2	88.5	5.5	8	11
	60	6.2	5.4	93.7	6.2	8	10
	95	6.6	5.7	98.2	7.0	8	6
0.01	0	9.6	4.2	52.2	4.0	3	2
	30	5.8	5.1	60.0	4.4	9	6
	60	5.8	5.0	61.0	4.4	7	6
	95	6.4	5.2	75.7	5.3	8	2
0	0	7.0	2.9	28.9	2.7	0	0
	30	5.7	3.5	41.3	3.2	5	2
	60	5.2	3.2	40.0	3.2	4	2
	95	6.4	3.1	40.0	3.0	4	1

gation was less marked on fragments excised from the lower regions of the hypocotyl. The breadth of the fragments increased greatly in the presence of IAA, this effect being greatest on the 0.1 and 1.0 mg./l. concentrations. The increase in breadth was due in part to the formation of the

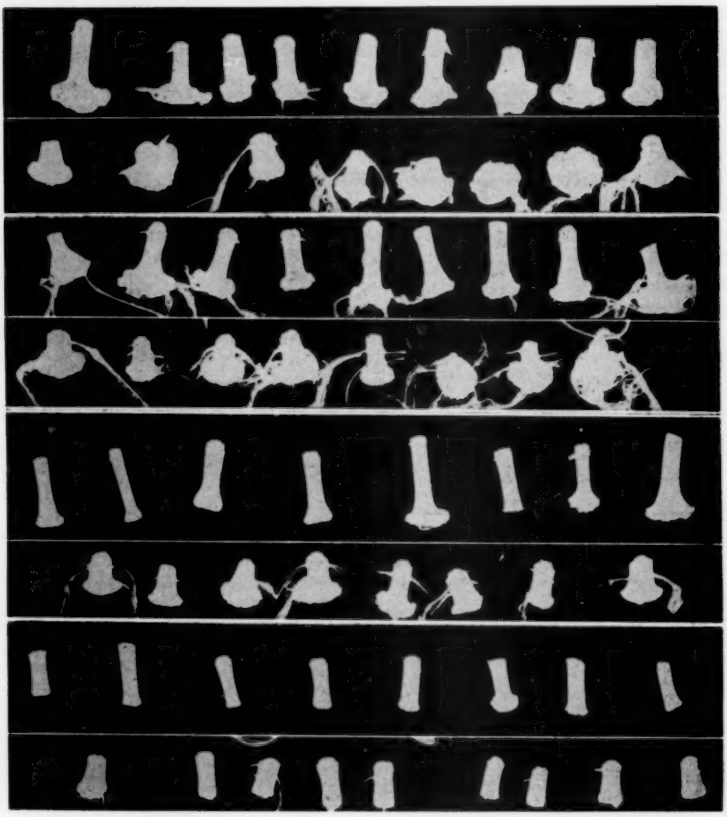


FIG. 2. Fragments of sunflower hypocotyl cultured on nutrient agar containing 1.0 mg./l. IAA (rows 1 and 2), 0.1 mg./l. IAA (rows 3 and 4), 0.01 mg./l. IAA (rows 5 and 6), 0 mg./l. IAA (rows 7 and 8). Fragments in upper of each pair of rows excised 0 mm. from point of attachment of cotyledons. Fragments in lower row excised 95 mm. from this point.

characteristic hyperhydric tissue which has already been described for sunflower stem fragments cultured in the presence of these concentrations of IAA (1). Fragments from levels 60 mm. and 95 mm. sometimes became almost spherical in outline as a result of this growth (fig. 2). In the group of fragments from the apex of the hypocotyl, the swelling was confined to

the surface of the fragment in contact with the agar. Maximum adventitious root production occurred on those fragments which were in contact with 0.1 mg./l. IAA. The mean number of such roots was greatest in fragments on levels 30 and 60 mm. and least on levels 0 and 95 mm. Production of adventitious roots occurred mainly at two regions on the fragments in contact with IAA, one group arising near the radicle end of the fragment, the other from the end in contact with the agar (fig. 2).

The IAA brought about a considerable increase in both fresh and dry weights of these fragments, the greatest increase occurring in the presence of 1 and 0.1 mg./l. of this substance. IAA also eliminated the difference in weight between fragments from level 0 and those from lower levels which was considerable in those fragments cultured in the absence of IAA.

The aim of these studies was to determine the relationship between the capacity for growth of isolated fragments of sunflower hypocotyl and the region of this organ from which they were excised. Capacity for elongation was localized mainly in the 10 mm. of the hypocotyl immediately below the point of attachment of the cotyledons. This capacity was enhanced both by inoculating the fragment with crown-gall bacteria and by culturing the fragment in the presence of 0.01 mg./l. IAA. Fragments from the upper region of the hypocotyl, when cultured on sucrose and mineral agar, had a smaller capacity for growth in breadth and in fresh and dry weight than had those taken from the lower regions. This difference was eliminated when 1.0 or 0.1 mg./l. of IAA was present in the medium. Inoculation with crown-gall bacteria also largely eliminated these differences. Capacity for production of adventitious roots was lowest in fragments taken from the upper 10 mm. of the hypocotyl. Root production by these fragments was enhanced by the presence of 0.1 mg./l. IAA in the medium but still remained less than that of fragments from the central region grown on a similar medium. Inoculation with crown-gall bacteria also enhanced root production. Fragments from the extreme base of the hypocotyl developed fewer roots than did fragments from the central region. Capacity for tumor production in response to inoculations with crown-gall bacteria was about equal in all fragments.

This work was done in part on a grant in aid recommended by the Committee on Growth of the National Cancer Society.

NEW YORK BOTANICAL GARDEN
NEW YORK, NEW YORK

LITERATURE CITED

1. DE ROPP, R. S. The response of normal plant tissue and of crown-gall tumor tissues to synthetic growth hormones. Amer. Jour. Bot. **34**: 53–62. 1947.
2. SACHS, J. Textbook of Botany. Oxford Univ. Press, New York. 2nd Ed. 1882.

SOME EFFECTS OF PHOSPHORUS ON THE DEVELOPMENT, THE B VITAMIN CONTENT, AND THE INORGANIC COMPOSITION OF OATS

THOMAS A. MCCOY, DAVID G. BOSTWICK[1] AND A. CHARLES DEVICH

Received March 10, 1951

The chemical composition of various grasses has been the subject of much research. Most of this work has been directed toward the influence of environment factors on the nutritive content of the plant. While it is common knowledge that temperature and light markedly affect the composition of plants, an equally important aspect is the influence of inorganic ions on the content of plant tissue. The principal constituents which have been studied to date include carotene, ascorbic acid, and the inorganic ions. Of the work done on the effects of major elements, the main emphasis has been on the study of plant tissue grown in nutrient solutions completely devoid of one element with very little work done on the effect of high and low concentrations of specific inorganic ions.

Recently a number of articles have been published suggesting a relationship between the fertility of soils and human and animal nutrition. Before any positive conclusions are justified as to the mechanism of this relation, a comprehensive study of the effect of varying concentrations of inorganic ions (available to the plant) on the nutritive content of plants will be necessary. The work described in the present paper was designed to study the effect of high and low concentrations of phosphorus on the folic acid, biotin, niacin, calcium pantothenate, and inorganic content of oats.

Experimental procedure

Wintok Oats no. CI-3424 from the Oklahoma Agricultural Experiment Station were the plants used in this study. The seeds were germinated in flats containing pure quartz sand which had been previously leached with hydorchloric acid and washed with distilled water. The seeds were planted in flats, then watered with distilled water. When 11 days old the seedlings were transplanted into pure quartz sand in four-inch glazed soil jars, and the different treatments were watered with their respective nutrient solutions. The following watering systems was used: Approximately 100 ml. of nutrient solution was introduced into each pot every day. Every fourth day the pots were flushed with distilled water to prevent undue salt accumulation.

There were ten pots to each treatment with two plants per pot. The compositions of the nutrient solutions were based on Solution 1, Method B, of HOAGLAND and ARNON (6) and are listed in table I. The nutrient solu-

[1] Deceased, November 19, 1950.

tions were kept in 18 liter bottles which had been painted with aluminum paint to prevent algal growth. When the solutions were prepared, care was taken to control the pH of these solutions to prevent the precipitation of phosphate ions with the alkaline earths. There were five treatments in which the concentrations of phosphorous were 5, 2, 1, $\frac{1}{4}$, and $\frac{1}{10}$ times that of the control concentration. Sodium chloride was added to produce the same osmotic pressure in all nutrient solutions. In addition to the regular control, another control which contained no sodium chloride was employed in order that the results could be compared with previous work of this and other laboratories. For the purposes of this paper, the different treatments will be referred to in the following manner: 5P, 2P, P, P/4, P/10, and the H-A control.

The seeds were planted April 1, 1949, and at the end of three weeks a definite phosphorus deficiency was noted in the P/10 treatment. No distinct visual differences were observed in the other treatments. During the latter part of May, the P/4 and P/10 plants began shooting; and by June

TABLE I

COMPOSITION OF THE NUTRIENT SOLUTIONS IN MILLIMOLES PER LITER

Treatment	KNO_3	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	NaH_2PO_4	NaCl	KCl
5P	5.0	5.0	2.0	1.00	4.0	1.0	....
2P	5.0	5.0	2.0	1.00	1.0	4.0	....
P	5.0	5.0	2.0	1.00	....	5.0	...
P/4	5.0	5.0	2.0	.25	...	5.0	.75
P/10	5.0	5.0	2.0	.10	...	5.0	.90
H-A	5.0	5.0	2.0	1.00	...	....	....

6 inflorescences had appeared on the P/10 plants. All the plants in this treatment had flowered by June 13.

The P/4 plants began flowering on June 13, and by June 18 all of these plants had flowered. There were no differences in time of shooting of the 5P, 2P, and P plants, and they all began flowering on June 18. The H-A control did not begin flowering until June 22. The low phosphorus treatments were slightly taller than the remaining groups, but the stems were rather spindly. General observations of the plants indicated the 2P, P, and the H-A control were the most vigorous plants. The 5P plants did not appear as vigorous as the remaining groups although they did not show any definite visual differences other than general appearance. The plants were harvested June 29, 1949. The harvesting consisted of cutting the plants at the first node and cleaning the tops. The plants were brought into the laboratory and were dried in a oven at 70° C for eight hours and at 105° C for sixteen hours. Afterwards the plant material was weighed and ground in a small Wiley mill using a 60-mesh sieve. The ground material was allowed to equilibrate with the moisture in the air, and calculations were made to correct the residual moisture content of the tissues.

The methods of analysis and preparation of the plants for these anal-

yses were the same as described previously (**9**) with the following additions: Folic acid was determined by the method of RABINOWITZ and SNELL (**15**), biotin was determined by the method of WRIGHT and SKEGGS (**20**), and calcium pantothenate was determined by the method of McCOY and SNYDER (**10**). The dry weights and the results of these analyses are listed in table II.

Results and discussion

The control plants (P and the H-A) grown in complete nutrient solution were completely normal with respect to outward appearances. The

TABLE II

COMPOSITION OF OATS GROWN WITH DIFFERENT NUTRIENT SOLUTIONS

Treatment	Dry weight gm.	Contents of elements, dry weight basis						Vitamin content, γ/gm. dry weight			
		Ca	Mg	K	Na	P	N	Folic acid	Biotin	Niacin	Calcium pantothenate
		%	%	%	%	%	%				
5P	13.02	.34	.42	3.27	1.16	.95	3.54	1.56	.183	61.1	33.7
2P	13.68	.62	.41	3.18	1.14	.34	3.30	1.16	.168	60.1	28.5
P	14.38	.75	.67	3.50	1.12	.26	3.19	1.05	.164	57.6	30.2
P/4	13.90	.90	.66	4.04	1.30	.20	3.02	1.11	.148	56.2	26.1
P/10	8.95	.80	.53	3.59	.95	.12	2.30	1.02	.102	48.5	21.5
H-A	13.30	.98	.77	4.18	.25	.25	3.16	.93	.146	59.7	29.1
T_{05} S_x	1.54	.06	.75	.75	.09	.02	.11	.143	.039	4.9	1.89
n	44	43	43	44	41	42	44	41	43	44	44

P/10 plants were definitely lighter in weight and showed slight phosphorus deficiency symptoms.

It was noted that there was no appreciable difference in the amount of tillering among the 2P, P, and the H-A control series. The P/4 plants had three fourths as many tillers and the P/10 plants had less than half as many as the controls. This effect has been well demonstrated by TEAKLE (**16**), McCLELLAND (**8**), and others. However, Teakle found that when wheat was receiving an adequate amount of phosphorus, a variation of phosphorus in the nutrient solution (one to 50 parts per million) had no appreciable effect on the amount of tillering. In the present study, the 5P plants had more tillers than the control plants, but this difference could not be considered statistically significant. DICKSON (**4**) reported that when oats received one tenth the normal amount of phosphorus there was no tillering in the plants. While the plants in the present experiment showed a similar tendency, nevertheless, the oat plants receiving one tenth the control amount of phosphorus did tiller to some extent.

The effect of phosphorus on the time of appearance of inflorescences is worthy of note. There have been many conflicting reports on the effects of mineral nutrition on the development of plants. NAYLOR (**12**) observed that in *Xanthium pennsylvanicum* mineral deficiencies retard the rate of

development. MILLER (11) stated that, in general, phosphate deficiencies will retard the time of maturity. WITHROW (19) reported that external nitrogen supply altered the time of development in some species but not in others.

Cajlachjan, according to WHYTE (18), stated that while the conditions of mineral nutrients cannot alone lead to the initiation of sexual maturity in a plant it can become an important factor in plant development when the plant is developing under a favorable photoperiod. He classified all plants into three groups, *i.e.*, nitro-negative, nitro-positive, and nitro-neutral, on the basis of the effect of inorganic nutrients on the time of maturity (primarily on a nitrogen nutrition basis). In his classification he included oats (a long-day plant) in the nitro-negative plants which attained flowering sooner when given little nitrogen. These nitro-negative plants produced inflorescences at an earlier date when subjected to a sub-optimum balance of inorganic ions. His classification of Xanthium as a nitro-positive plant agrees with the results of NAYLOR (12). DICKSON (4) reported that the period of development was reduced by one week when the concentration of nitrogen or phosphorus was reduced to one tenth that of his control. The results of the present work are in accord with Dickson's findings. NOLL's statement (13), that an increase in phosphorus above the needs of the crop will not hasten the date of maturity, was also confirmed.

In considering the analytical results listed in table II, it is interesting to note that the calcium content of the tissues increased as the amount of phosphorus decreased from 5P to P/4. The P/10 treatment had practically the same amount of calcium as the P group. Further, the H-A control, which had no appreciable amount of sodium, contained a significantly larger amount of calcium when compared with any of the other groups. DICKSON (5) reported that the decrease of phosphorus to the one tenth level reduced the amount of calcium to approximately 53% in the grain and to 55% in the straw, an opposite tendency to that noted in the present study. WATSON and NOGGLE (17) in a study of deficiencies in oats reported that calcium content remained the same in the leaves and increased somewhat in the stems when the plants were subjected to a nutrient solution devoid of any phosphorus. Previous work (9) from this laboratory indicated that oats receiving one fourth the amount of phosphorus contained slightly more calcium than the control. The magnesium content of the tissues was lower in the 5/P and 2/P groups and higher in the H-A group when compared to the other plants. The potassium content of the plants was higher in the P/4 and the H-A control groups lower in the 5P and 2P groups. OWEN (14) reported that a phosphorus-deficient plant contained less potassium in the foliage and that application of phosphorus to the soil increased the potassium content. WATSON and NOGGLE (17) observed that phosphorus-deficient oats contained more potassium in the leaves and roots when compared to control plants. The present results are in agreement with those of Watson and Noggle. The amount of sodium in the plant tissues was

essentially the same in the 5P, 2P, and P groups. It was lower in the P/10 and higher in the P/4 groups.

The phosphorus content followed rather closely the corresponding treatments of the nutrient solution but not in the same ratio. This element and nitrogen were the only two elements studied whose content compared closely between the P and the H-A groups. There seemed to be a definite relationship between the amount of nitrogen and the amount of phosphorus absorbed. The 5P plants, which contained the largest amount of phosphorus, contained the largest amount of nitrogen; and the P/10 plants contained the smallest amount of nitrogen. Further, as the phosphorus content of the tissues increased between treatments, the nitrogen content also increased. This phenomenon is contrary to some previous reports. For example, MacGillivray (**7**), Colby (**3**), Carolus (**1, 2**), and several other workers have observed an inverse relationship between the phosphorus and nitrogen absorption by various plants. However, Watson and Noggle (**17**), working with young oats, showed that a phosphorus deficiency resulted in a decrease in nitrogen in the leaves, stems, and roots. Further, Cajlachjan's classification of plants (with respect to the relation of mineral nutrients to the time of flowering) is based on nitrogen nutrition; and in his extensive work on oats, he reported that a phosphorus deficiency hastened the time of flowering. This observation can explain the relation of phosphorus and nitrogen and their effect on time of maturity. It appears that in oats there is a direct relation between the absorption of phosphorus and nitrogen. If, while developing under a favorable photoperiod, an oat plant receives a reduced amount of phosphorus, then a smaller amount of nitrogen is absorbed and the period of development is shortened. There is a possibility that the effect of chloride ion on the absorption of nitrate ion could explain this phenomenon; but since the nitrogen content of the H-A group was practically the same as the nitrogen content of the P plants, this possibility would seem unlikely.

The folic acid content of the 5P treatment was significantly higher than that of any of the other treatments. It appears that, when an excessive amount of phosphorus is available to the plant, the folic acid concentration of the plant tissues increases. The results of the analysis of biotin showed a very similar trend. However, this trend is more consistent with the phosphorus and nitrogen content of the plant tissues. The niacin results showed less difference among the treatments than any of the other B vitamins studied. While the higher phosphorus treatments did contain a consistently larger amount of niacin per gram dry weight, these differences were not significant. The P/10 treatment was the only treatment in which the plants had a significantly lower amount of niacin. As in the case of folic acid and biotin, the calcium pantothenate content was largest in the 5P treatment and smallest in the P/10 treatment. The H-A plants did not differ from the P group with respect to niacin and calcium pantothenate. They did contain slightly less biotin and folic acid than any of

the P plants. However, these differences were not statistically significant at the 5% level. In all cases, there appeared to be a direct relation between the phosphorus content of the tissue and the concentration of folic acid, biotin, niacin, and calcium pantothenate.

In the 2P, P, P/4, and H-A control plants, the vitamin contents did show a trend with respect to the phosphorus content but the differences were not statistically significant. This confirmed the observation that the inorganic nutrition of plants does not affect the vitamin content as markedly as other environmental factors. Further, it should be mentioned that changes in plant composition due to changes in the total phosphorus content of the substrate would be far less for soils than for sand culture. The plants receiving five times the control amount of phosphorus contained the largest amount of vitamins on the basis of dry weight and the highest percentage of phosphorus and nitrogen. At the same time, this group contained less calcium and magnesium. These effects on vitamin content may be attributed to the nitrogen nutrition of the plants and may be related to the varying concentrations of phosphorus in the nutrient solutions only through its effect on the absorption of nitrogen.

Another interesting point is that plants grown in the H-A solution contained significantly greater amounts of calcium, magnesium, and potassium. This could be due to the presence of sodium in the other treatments. The present work indicated that sodium possibly affected the absorption of the cations, but that chloride did not affect the absorption of the anions.

This experiment was repeated exactly one year later to check the phosphorus-nitrogen ratio and its effect on development of the plant. The results were in agreement with the previous work.

Summary

Wintok Oats no. CI-3424 were grown in sand culture with the amount of phosphate-phosphorus varying from five times to one tenth that of the control. It was noted that phosphorus-deficient plants matured earlier than the other treatments. An explanation of this fact was offered on the basis of nitrogen nutrition. In addition, a direct phosphorus-nitrogen relation was demonstrated in the plants. It was noted that the plants receiving five times the control amount of phosphorus contained more and the plants receiving one tenth the amount of phosphorus contained less folic acid, biotin, niacin, and calcium pantothenate in the plant tissue. The other plants had practically the same vitamin content, except the control group, which had no sodium chloride, contained less folic acid than any of the other treatments.

The authors would like to express their appreciation for the cooperation and assistance of the laboratory staff of The Samuel Roberts Noble Foundation.

THE SAMUEL ROBERTS NOBLE FOUNDATION
ARDMORE, OKLAHOMA

LITERATURE CITED

1. Carolus, R. L. Experiences with rapid chemical tests for the determination of nutrient deficiencies in vegetable crops. Proc. Amer. Soc. Hort. Sci. **33**: 579–583. 1935.
2. Carolus, R. L. The relation of potassium, calcium, and sodium to magnesium deficiency. Proc. Amer. Soc. Hort. Sci. **33**: 595–599. 1935.
3. Colby, H. L. Effect of starvation on distribution of mineral nutrients in French prune trees grown in solution culture. Plant Physiol. **8**: 387–394. 1933.
4. Dickson, J. G. The value of certain nutritive elements in the development of the oat plant. Amer. Jour. Bot. **5**: 301–324. 1918.
5. Dickson, J. G. The relation of certain nutritive elements to the composition of the plant. Amer. Jour. Bot. **8**: 256–272. 1921,
6. Hoagland, D. R. and Arnon, D. I. The water-culture method for growing plants without soil. California Agr. Exp. Sta., Cir. no. **347**. 1938.
7. MacGillivray, J. H. The importance of phosphorus in the production of seed and non-seed portions of a tomato fruit. Proc. Amer. Soc. Hort. Sci. **22**: 374–379. 1926.
8. McClelland, C. K. Effects of minor plant foods on growth activities and development of oats. Jour. Amer. Soc. Agron. **23**: 304–311. 1931.
9. McCoy, T. A., Free, S. M., Langston, R. G., and Snyder, J. Q. Effect of major elements on the niacin, carotene, and inorganic content of young oats. Soil Sci. **68**: 375–380. 1949.
10. McCoy, T. A. and Snyder, J. Q. The use of *Streptococcus faecalis*, A.T.C.C. no. 6057, as a test organism in microbiological assay. Trans. Oklahoma Acad. Sci., in press (1950).
11. Miller, E. C. Plant Physiology. McGraw-Hill Book Company, 2nd Ed., New York, 1938.
12. Naylor, A. W. Effect of nutrition and age upon rate of development of terminal staminate inflorescences of *Xanthium pennsylvanicum*. Bot. Gaz. **103**: 342–353. 1941.
13. Noll, C. F. The effects of phosphate on early growth and maturity. Jour. Amer. Soc. Agron. **15**: 87–97. 1923.
14. Owen, O. The analysis of tomato plants. II. The effect of manurial treatment on the composition of tomato foliage. Jour. Agr. Sci. **21**: 442–451. 1931.
15. Rabinowitz, J. C. and Snell, E. E. The vitamin B_6 group. XI. An improved method for assay of vitamin B_6 with *Streptococcus faecalis*. Jour. Biol. Chem. **169**: 631–642. 1947.
16. Teakle, L. J. H. The absorption of phosphate from soil and solution cultures. Plant Physiol. **4**: 211–232. 1929.

17. WATSON, S. A. and NOGGLE, G. R. Effect of mineral deficiencies upon the synthesis of riboflavin and ascorbic acid by the oat plant. Plant Physiol. **22**: 228–243. 1947.
18. WHYTE, R. O. Crop Production and Environment. Faber and Faber, Ltd., London. 1949.
19. WITHROW, A. P. The interrelationship of nitrogen supply and photoperiod on the flowering growth and stem anatomy of certain long-day and short-day plants. Bulter Univ. Bot. Stud. **7**: 40–64. 1945.
20. WRIGHT, L. D. and SKEGGS, H. R. Determination of biotin with *Lactobacillus arabinosus*. Proc. Soc. Exp. Biol. & Med. **56**: 95–98. 1944.

AUTORADIOGRAPHY AS AN AID IN DETERMINING THE GROSS ABSORPTION AND UTILIZATION OF FOLIAR APPLIED NUTRIENTS[1]

S. H. Wittwer and W. S. Lundahl

(WITH THREE FIGURES)

Received March 26, 1951

Favorable growth responses have recently been obtained with some phosphorus compounds applied as foliar sprays to vegetable crops, the objective being to supplement the usual supply of phosphorus absorbed by the roots (**3**). Preliminary studies utilizing a 25 millimolar solution of phosphoric acid enriched with small quantities of radioactive phosphorus indicated that the nutrient was absorbed by the leaves of a wide variety of crops and within a few hours translocated to all plant parts, particularly to meristematic regions.

A technique for making autoradiograms relating to root absorption and subsequent distribution of phosphorus in tomato fruit and leaves at various stages of development has been outlined (**1**). Suggestions for improving the quality of autoradiograms of biological materials have been described by Boyd (**2**). As a means of demonstrating the rapidity of absorption, translocation, and selective utilization of foliar applied nutrients, autoradiography offers a unique approach. The effects of chemical agents and variations in the external environment on nutrient absorption can readily be demonstrated. Autoradiograms provide one of the best means of obtaining indications of relative metabolic activities of various plant tissues since their accumulative capacities for many of the foliar applied nutrients is clearly revealed and located. Herein is presented a method of simultaneously preparing a large number of autoradiograms of entire plants, such that the intensity of the images is within limits proportional to the concentration of the radioactive material in the tissues derived from various applications of radio-phosphorus. Autoradiograms are included which illustrate some results obtained.

Preparation of autoradiograms

Bean, corn, and transplanted tomato seedlings were grown in four-inch clay pots of sand and supplied with a minus phosphorus nutrient solution. Reserves of phosphorus in the seeds of bean and corn and in the transplanted tomato plants were in most instances sufficient for seedling growth. When of the appropriate size to occupy an area of contact film having dimensions of 8×10 inches, the foliage or selected parts thereof were treated by momentarily dipping into a 25 millimolar solution of *o*-phosphoric acid

[1] Journal Article no. 1233 of the Michigan Agricultural Experiment Station.

which assayed 1 micro-curie of P^{32} per milliliter. In some instances specific quantities of the solution were applied to certain vegetative tissues or, for contrast, diluted with distilled water and added to the root cultures. However, for comparable expression of results the above ratio of P^{31} and P^{32} was the same in all treating solutions. Dreft was added as a wetting agent at the rate of 0.2%.

At various time intervals following treatment, entire plants were harvested, and the roots carefully removed and washed free of sand. An attempt was made to remove residual and adsorbed radio-phosphorus on the treated surfaces by successive washing in 25 millimolar solutions of *o*-phosphoric acid and distilled water, respectively, until the washing solutions gave no additional count above background. Excess water was taken up with blotting paper. The entire plant was then placed between 8×10 inch sheets of botanical pressing paper. The various sheets of paper containing the plant specimens were then layered between 8×10 inch masonite boards and previously heated steel plates which were one-fourth or one-half inch in thickness. The layered pile of plant material, pressing paper, masonite boards, and steel plates was placed under several 250 watt infrared heat lamps for rapid drying. Several one-half inch thick 8×10 steel plates were placed on top of the layered stack of plant specimens to facilitate pressing during drying. The pressure of the plates during drying resulted in smooth plant surfaces essential for uniform film contact. The entire interval between harvesting and final drying of the plant tissue was no more than one hour.

Following drying, the plant specimens were allowed to cool and then carefully lifted from the original pressing paper and placed on new sheets. Removal of the plant from the original pressing paper was necessary to avoid smears on the autoradiograms induced by plant juices which may be exuded during the pressing-drying process. Because of the rapidity of the drying process (one hour), movement of the nutrient within the plant during processing was minimized. The new paper with the mounted specimen was then covered with a protective sheet of thin pliofilm held firmly against the plant with Scotch tape. This served to keep the sample in place, preserve it for future reference, and prevent scratching of the photographic film which was placed against it. In order to assure uniform contact with the emulsion, a second sheet of botanical specimen paper was placed on top of the film and the entire preparation mounted between two masonite boards. A special light-proof wooden (white pine) box was constructed to accommodate a number of such units. This exposure box was provided with an adjustable inner steel plate such that, when the preparations were stacked inside with one-half inch steel plates between each unit for shielding and pressure, it could be forced down on the layered stack of specimens by turning a vise handle on the box cover. The film used was 8×10 inch Kodak No-Screen X-Ray, which was exposed for five to seven days and developed for seven minutes in X-Ray developer. No fogging of the emul-

sion resulting from the possible presence of peroxides of terpenes in the wood of the exposure box was evident.

Results

Figures 1, 2, and 3 are illustrative of some of the results obtained. The intensity of the autoradiograms of the plants shown are comparable and representative of the relative quantities of the radio-phosphorus absorbed and mobilized in various tissues, since they were all prepared simultaneously by the methods outlined above. Because of apparent differences in

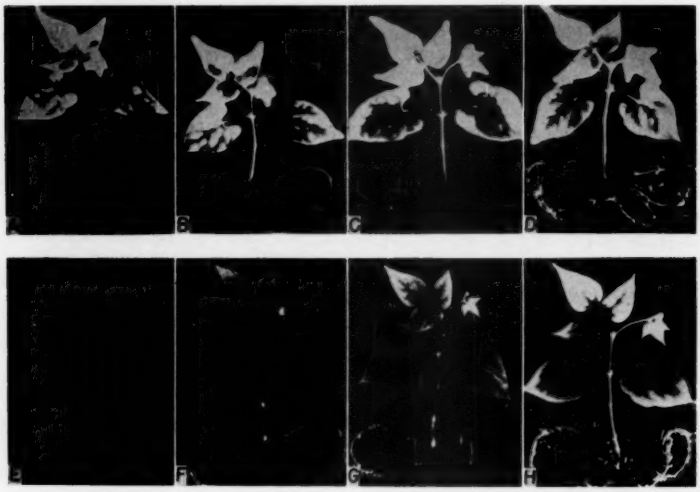


FIG. 1. Autoradiograms of bean plants demonstrating foliar (A to D) and root (E to H) absorption of radioactive phosphorus. From left to right, the autoradiograms were made from plants harvested two, six, 24, and 48 hours following treatment. The apparent distribution of radio-phosphorus in the leaves may not necessarily indicate its precise histological location before processing. Lack of continuity in the roots results from minor breakages and the higher concentrations in the root tips.

rates of leaf absorption and utilization of the radio-phosphorus, some of the autoradiograms, especially those in fig 2, were necessarily over-exposed.

In figure 1, the relative utilization of foliar (A to D) and root (E to H) sources of applied radioactive phosphorus in beans with the progression of time is illustrated. From left to right, the autoradiograms represent plants harvested at two, six, 24, and 48 hours subsequent to treatment. With the foliar applications approximately 0.5 micro-curie of P^{32} was applied to the leaf system of any given bean plant. Root applications consisted of five micro-curies in 100 milliliters of distilled water supplied to each plant grown in a minus phosphate sand culture in a four inch pot. Thus, the specific activity of the phosphate upon addition to the sand cultures was not

changed. Approximately 10 times as much radio-phosphorus was applied per plant in the root applications. The relatively greater efficiency of foliar absorption, on the basis of the amounts applied, is suggested. It can also be noted that insofar as time is concerned, the translocation of the nutrient from the leaves to the roots appears to be as rapid as from the roots to the leaves with the concentrations used. This has also been demonstrated by tissue counts and time activity curves using similar plant material, and will

FIG. 2. Utilization of foliar applied radioactive phosphorus in the tomato plant as revealed by autoradiograms. In A and B the entire foliage was momentarily dipped in the radioactive phosphorus and the plants harvested after two and 24 hours, respectively. In C and D only the second and third true leaves were treated and the plants similarly harvested after two and 24 hours, respectively.

be published elsewhere. In all autoradiograms the light portions in the plant tissues designate the presence of absorbed radio-phosphorus. Residual and adsorbed phosphorus was removed before the plants were prepared for film exposure. It can be noted in the autoradiograms of figure 1 A and B, taken of plants harvested two and six hours after treatment, that the younger leaves show the greatest concentration of the radio-phosphorus. This suggests that not only do the young meristematic tissues most readily

utilize available phosphorus but that they are most efficient in its absorption. The greater concentration of phosphorus in the young leaves, lateral buds, nodes, root tips, and root nodules follows a similar pattern whether the nutrient is supplied to the foliage or the roots. The gross distribution of radio-phosphorus in the leaves and the conductive tissues therein apparently depends on the age of the leaves, the time following treatment that the autoradiograms were prepared and whether leaf or root applications were employed.

It is clearly demonstrated in the autoradiograms of the tomato plants of figure 2 that foliar applied phosphorus is selectively mobilized by certain

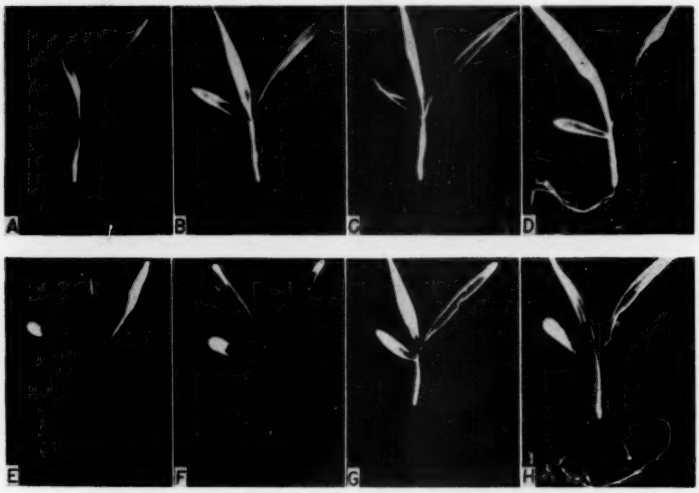


FIG. 3. Autoradiograms of corn plants showing the effect of foliar placement on the absorption by the leaves of radioactive phosphorus. In A to D, the nutrient was placed in the funnel formed by the leaf sheaths of the meristematic leaves of the growing tip. The autoradiograms E to H represent plants whereon only the outer portions of the leaves were treated. From left to right the plants were harvested in both groups at two, six, 24, and 48 hours subsequent to treatment.

tissues. In A and B all the leaves were momentarily dipped into the active solution while with C and D only the second and third true leaves, counting from the bottom of the plants, were treated. The two autoradiograms on the left (A and C) were taken from plants harvested two hours after treatment while those on the right (B and D) represent plants harvested 24 hours subsequent to receiving the radioactive phosphorus. In contrast to the bean plants, it can be noted in the tomato that within two hours of treatment, the phosphorus was absorbed by the leaves and translocated to the roots. Movement to the roots was more rapid than to the vegetative growing tips as is demonstrated in C. It is of further interest to note that after 24 hours (D), the foliar applied radio-phosphorus had accumulated

in the root crown, and in the vegetative-growing tip and young leaves, with none apparently moving into the non-treated mature leaves.

The effect of foliar placement of radio-phosphorus on its rapidity of absorption by the leaves of the corn plant is illustrated in figure 3. Autoradiograms A and D were taken of plants harvested at two, six, 24, and 48 hours subsequent to the application of one-fourth micro-curie of P^{32} in the funnel of the growing tip formed by the leaf sheaths, while autoradiograms E and H were developed from plants harvested at the same intervals of time but with only the expanded outer portions of the leaves dipped in the usual solution assaying 1 micro-curie P^{32} per milliliter. The funnel formed by the young leaf sheaths in the center of the growing corn plant consists of meristematic leaf tissue, which provides an advantageous location for applying nutrient solutions. As can be seen in figure 3 A, translocation to the roots and to non-treated leaf portions occurs within two hours after treatment if the radioactive phosphorus is applied in the natural funnel formed by the meristematic leaf sheaths.

Summary

Autoradiography offers a means of obtaining visual records of the gross absorption, translocation and selective mobilization of foliar applied nutrients.

A method of simultaneously preparing a large number of autoradiograms by exposure of 8×10 inch Kodak No-Screen X-Ray film to properly prepared plant specimens is described.

Autoradiograms of bean, corn, and tomato plants demonstrate that leaves are efficient organs for absorption of radio-phosphorus. Subsequent distribution in the bean plant of the foliar applied radionuclide is comparable to that taken up by roots. The relatively greater nutrient absorption efficiency of the younger expanding leaves is suggested.

The authors gratefully acknowledge the many helpful suggestions by Dr. L. F. Wolterink in the initial phases of this study.

Department of Horticulture and Department of Biological Science
Michigan State College
East Lansing, Michigan

LITERATURE CITED

1. Arnon, D. I., Stout, P. R., and Sipos, F. Radio-active phosphorus as an indicator of phosphorus absorption of tomato fruits at various stages of development. Amer. Jour. Botany **27**: 791–798. 1940.
2. Boyd, G. A. The physical principles and techniques of autoradiographs. U. S. Atomic Energy Commission Publication MDDC-1226. 1947.
3. Silberstein, O. and Wittwer, S. H. Foliar application of phosphatic nutrients to certain vegetable crops. (In press.) Proc. Amer. Soc. Hort. Sci. 1951.

NITROGEN AND AMINO ACID CONTENT OF THE VARIOUS PARTS OF *ANDROPOGON ISCHAEMUM* L.

GLENN S. RABIDEAU AND MARVIN B. EDWARDS

(WITH THREE FIGURES)

Received April 30, 1951

Introduction

Knowledge of the nitrogen metabolism of plants is of importance for an understanding of the growth and development of the plants themselves and for their evaluation as food for grazing animals. Of particular significance with respect to the latter use is the amino acid composition of the grass proteins. Although a few studies have been made of the amino acid composition of grass proteins (CHIBNALL, **2**; LUGG, **8**) no studies have ever been made of the amino acid compostion of grasses of range importance in the Southwest. FUDGE and FRAPS (**5**) have, however, made a fairly extensive study of the total nitrogen composition of certain Texas grasses. A somewhat more limited study involving rye grass, along with certain cereal grasses, has been made by MACVICAR (**9**). A study of the amino acids in alfalfa made by STEWARD *et al.* (**11**), employing the chromatography method, indicated that several amino acids in addition to the amides, glutamine and asparagine, could be extracted with alcohol. This study further lists a number of amino acids which were detected after acid hydrolysis. Radioautography of amino acids containing S^{35} aided in the identification of various sulphur containing amino acids.

One of the most important considerations with respect to range grasses is the modification of nitrogen content associated with regrowth following grazing. The physiological effects of clipping certain pasture grasses to simulate grazing have been reported by LEUKEL *et al.* (**7**). Leukel and his co-workers concluded that frequent clipping reduces the carbohydrate and nitrogen content and favors vegetative rather than reproductive growth. A detailed study of the carbohydrate and nitrogen composition of the roots, stubble, and tops of clipped perennial rye grass plants was made by SULLIVAN and SPRAGUE (**13**). HOLSCHER (**6**) has reported decreased growth and dry weight in clipped western wheatgrass, *Agropyron Smithii* Rydb. and blue grama grass, *Bouteloua gracilis* (H.B.K.) Lag. BUKEY and WEAVER (**1**) made a similar study of little bluestem, *Andropogon scoparius* Michx., and big bluestem, *Andropogon furcatus* Muhl.

The present study deals with both total nitrogen and amino acid content of the King Ranch strain of *Andropogon ischaemum* L. The investigation was aimed, in part, at determining whether or not the amino acid content remains constant during the growth cycle.

Methods

Plants for this study were grown in sand culture in the greenhouse during the spring of 1950. The culture method and the sampling procedures have been set forth by TSAO *et al.* (**14**) in a discussion of the phosphorus nutrition of King Ranch bluestem. Plant samples for nitrogen and amino acids were collected separately but from the same set of cultures. Young plants were divided into roots and shoots, and older plants into roots, leaf tips, leaf bases, inflorescences, and tillers. These samples were dried in a forced air drying over at 75° C and then ground in a Wiley mill to pass a 60 mesh screen. Silica-free acid digests were prepared and analyzed for total nitrogen by the Koch and McMeekin method previously described by TSAO *et. al.* (**14**). Total nitrogen values obtained by micro-Kjeldahl distillation of occasional samples checked fairly closely with the Koch and McMeekin values.

Two types of amino acid determinations were made: (1) determination of free amino acids and the amides, glutamine and asparagine, from water extracts of dried material and (2) determination of free amino acids plus the amino acids derived from the acid hydrolysis of similar dried samples. Free amino acids were determined with 5 to 10 μl. aliquot portions of a water extract of dried sample, made by adding 15 ml. water to 0.2 gm. sample and allowing the material to stand for one half hour. The filtered extract was evaporated at room temperature and under reduced pressure to one ml. volume. STUART (**12**) has discussed the merits of the water extraction method for soluble nitrogen and claimed it to be superior to other solvent methods for this purpose. For the determination of free amino acids plus the amino acids derived from acid hydrolysis, an acid digest of 0.2 gm. of each dried plant sample was made by refluxing for 24 hours in 6 *N* hydrochloric acid. This digest was evaporated by means of heat and reduced pressure to a volume of one ml.

Amino acid composition of these samples was determined by the paper chromatography method. Preliminary chromatographic determinations indicated that several free amino acids in addition to the amides, glutamine and asparagine, were present in a water extract of unhydrolyzed dried plant material. Since certain of the spots occupied overlapping positions on the chromatogram, detection of some of these substances was impossible with one-dimensional chromatography. Because of this fact, the two-dimensional ascending chromatography method WILLIAMS and KIRBY (**15**) was used exclusively in this work. An aliquot portion of five μl. of either the water extract sample or the acid digest sample was applied to one corner of a sheet of Whatman #1 filter paper. Occasional 10, 25, and 50 μl. sample aliquots were used to check amino acids which occur in low concentration. The two developing agents were, first, water saturated phenol and, then, equal volumes of 2,4,6-collidine and 2,6-lutidine, mixed and saturated with water at room temperature. After drying the chromatograms in an oven, amino acid locations were made evident by spraying with ninhydrin reagent and redry-

ing with heat. Pure amino acid preparations were used in identification and concentration estimates. DENT'S (3) visual method for the estimation of concentrations was employed. Comparisons were made with chromatograms prepared with pure amino acids in a series of concentration ranges.

In an effort to evaluate the effects of simulated grazing upon total nitrogen and amino acid distribution, some plants were clipped 44 days after germination, and again 20 days later. The clipping removed the leaves at a height of about five cm. above the surface of the sand. When these plants were sampled, care was taken to distinguish between original structures and regrowth structures.

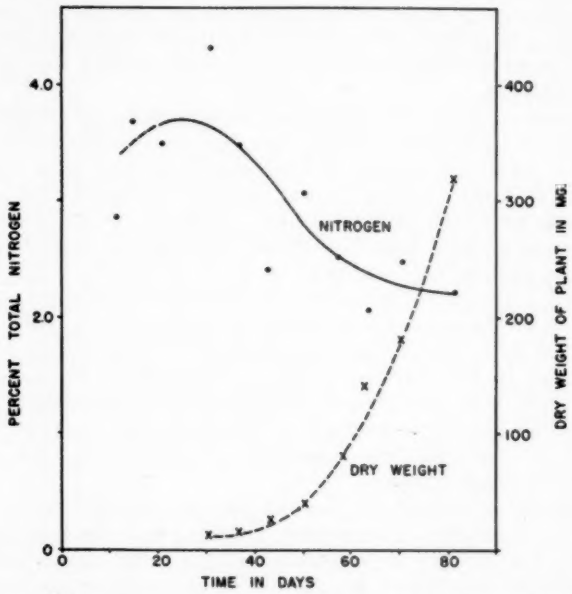


FIG. 1. Total nitrogen content of *A. ischaemum*. The figures represent weighted averages based upon the nitrogen content of the various plant parts. The dry weight per plant curve is superimposed for comparison.

Results

The total nitrogen percentages of the plants, on the whole plant basis, are shown in figure 1. The values are weighted averages of the nitrogen analyses of the plant parts. From about 30 days after emergence, there was a progressive decrease in percentage of total nitrogen as the plants increased in size. Results of total nitrogen analyses of various parts of these same plants are shown in figure 2. A decrease in the nitrogen percentage with age was noted in roots, leaf bases, leaf tips, tillers, and inflorescences; although this decrease was less rapid in roots and in leaf bases. The level of

nitrogen was about twice as high for tops and tillers as it was for roots and bases. The former decreased from about 4% at 30 days to about 3% at 80 days, whereas the latter decreased from 2 to 1.5% during the same interval. The inflorescences decreased from about 3.0 to about 2.0% total nitrogen during the 50 to 80 day period. While dry weights of whole plants were trebled during the 36 to 58 day period, total nitrogen values were decreased only approximately 33%. Therefore, during this period there was an actual increase in absolute nitrogen concentration on a per plant basis, ranging

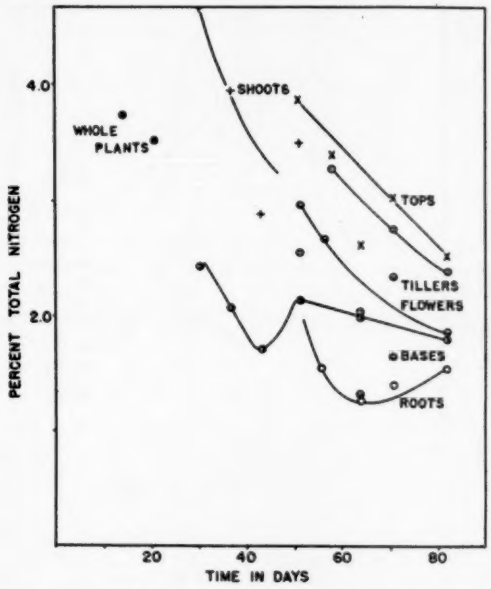


FIG. 2. The distribution of total nitrogen among the various plant parts.

from 0.7 mg. per plant at 37 days to 7.5 mg. per plant at 82 days. As is shown at the bottom of table II, results of total nitrogen determinations compare favorably with the calculated nitrogen percentage contents based upon the sums of the quantities of amino acids estimated by the chromatographic analyses.

The effects of clipping on the total nitrogen content of leaf tips, leaf bases, and roots are shown in figure 3. New leaf growth which followed clipping had, as would be anticipated, a relatively high nitrogen content. Clipping resulted, moreover, in an increase in nitrogen content of older leaf bases and of roots. This increase was followed within a week or two by a decrease in the percentage of nitrogen.

The distribution of free amino acids in the shoots is shown in table I.

The amount of free amino acid usually averaged about 10 to 25% of the amount of the same amino acid in the acid hydrolysates of comparable samples. Only alanine, aspartic acid and glutamic acid were found free in seed samples and then only in traces. In older plant samples, threonine and arginine were absent. Asparagine was not detected in the 31-day samples. Glutamine was detected only once, in the eight-day samples, and then only as a trace. A methionine derivative, probably the sulphoxide, since the hydrogen peroxide test of DENT (4) indicated the presence of sulphone, was

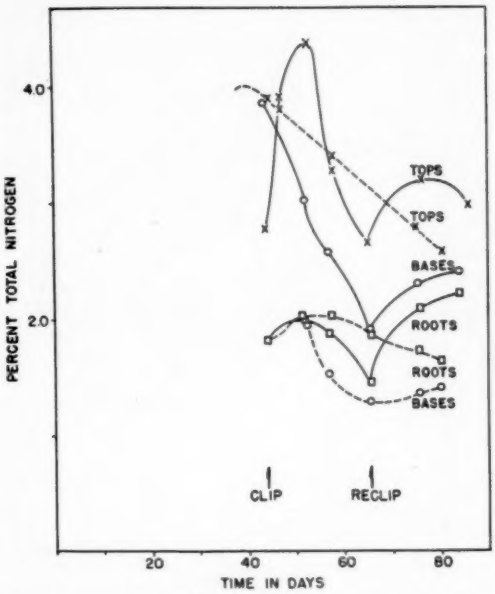


FIG. 3. The variation of total nitrogen following partial defoliation (solid lines). The curves for total nitrogen distribution in similar normal plant parts (dotted lines) are shown for comparison.

found in the free amino acid fraction. This finding is at variance with the results of STEWARD *et al.* (11) who found no evidence of methionine or its derivatives in the free amino acid fraction of alfalfa. According to DENT (4), methionine sulphoxide probably does not occur as such in plants but is formed as an oxidation product during the phenol development of chromatograms.

The distribution of amino acids derived from acid hydrolysis of shoot samples is shown in table II. The values are uncorrected for the small amount of free amino acids present. The most abundant amino acids derived from acid hydrolysis of shoot samples were leucine, alanine, histidine, serine, glycine, and lysine. These were followed in abundance by valine,

TABLE I

FREE AMINO ACIDS IN THE SHOOTS OF *A. ischaemum*. FIGURES ARE PERCENTAGE VALUES BASED ON DRY WEIGHT. THE AMINO ACIDS WERE DERIVED FROM AQUEOUS EXTRACTION AND CONCENTRATION AT ROOM TEMPERATURE AND UNDER REDUCED PRESSURE

Amino acid	Age in days						
	0 (seed)	8	11	15	21	26	31
Leucine	0	0.5	0.8	0.8	0.3	0.1	0.3
Valine	0	0.5	0.5	0.5	0.2	0.2	0.2
Alanine	trace	0.8	0.6	1.0	0.6	0.6	0.2
Threonine	0	0.1	0.1	0.1	0	0	0
Aspartic acid	trace	0.5	0.5	0.3	0.1	0.1	trace
Glutamic acid	trace	0.8	0.8	0.8	0.5	0.5	0.2
Lysine	0	0.5	0.5	0.5	0.3	0	0.2
Arginine	0	0.2	0.3	0.3	0	0	0
Methionine sulphoxide	0	0.5	0.6	0.6	0.2	0.2	0.2
Asparagine	0	1.3	1.0	0.5	0.3	0.1	0
Glutamine	0	trace	0	0	0	0	0

aspartic acid, glutamic acid, and proline, and finally by threonine. The percentages of these amino acids did not decrease markedly with increasing plant age.

Amino acids obtained by acid hydrolysis of other plant parts were similar to those of shoots.

TABLE II

AMINO ACIDS DERIVED PRIMARILY FROM ACID HYDROLYSIS OF SHOOTS OF *A. ischaemum*. FIGURES ARE PERCENTAGE VALUES BASED ON THE DRY WEIGHTS AND ARE UNCORRECTED FOR THE FREE AMINO ACIDS PRESENT BEFORE HYDROLYSIS. BLANK SPACES INDICATE THAT THE AMINO ACIDS WERE NOT DETECTED

Amino acid	Age in days							
	0 (seed)	8	11	15	21	43	58	82
Leucine	6.0	2.7	2.7	2.7	2.7	1.0	2.0	0.8
Valine	3.0	1.5	1.5	1.5	1.5	0.5	1.5	0.6
Alanine	3.2	1.8	1.8	1.8	1.8	1.0	1.5	0.7
Threonine	1.0	1.0	1.0	1.0	1.0	....	1.0	0.2
Histidine	3.0	4.0	2.0	2.0	2.5	....	1.0	....
Serine	3.2	4.0	2.5	2.5	2.5	....	1.7	1.0
Aspartic acid	2.4	2.5	1.3	1.5	1.5	0.2	0.3	....
Glutamic acid	3.0	1.6	1.8	1.8	1.8	1.7	1.7	1.0
Lysine	6.0	3.0	2.5	3.0	3.0	2.2	1.5	1.5
Arginine	6.0	3.0	2.0	2.0	2.5	1.3	1.5	1.0
Proline	3.0	2.0	2.0	2.0	1.5	....	....	....
Total	39.8	24.0	21.0	21.0	22.0	10.4	15.9	11.1
Percentage nitrogen (amino acids)	6.4	3.9	3.4	3.4	3.5	1.7	2.5	1.8
Percentage nitrogen (Nesslerization procedure)	5.1	3.0	3.9	3.3	3.0	2.9	2.4	2.5

An examination of the amino acid content of the various parts of plants at different sampling periods showed no significant changes.

Amino acid composition of the tops, bases, and roots of the plants which had been partially defoliated showed no change as to kinds of amino acids, but the increase in nitrogen percentage which followed clipping was reflected in increased amounts of amino acids in the new leaves. Neither an increase nor a significant decrease in the amino acids in leaf bases or roots accompanied the increase in amino acid quantity in newly formed leaves.

Discussion

The decrease in the percentage of total nitrogen with aging and increase in size of the grass plants is in agreement with the observations of other investigators. FUDGE and FRAPS (**5**) have reported that in several range grasses younger plants contain two to three times as much nitrogen on a percentage basis as older plants. The accumulation of a greater proportion of the total nitrogen in tops, tillers, and reproductive structures supports the general observation that younger, more actively growing plant parts are richer in nitrogen than older, less actively growing parts. The increase in nitrogen percentage in the shoots following removal of part of the leaf tissue is comparable to that found by SPRAGUE and SULLIVAN (**10**) working with orchard grass. HOLSCHER (**6**) has observed that repeated clipping ultimately reduces the percentage of total nitrogen in roots, leaf bases, and even newly formed leaves.

Percentages of free (water-soluble) amino acids detected in the shoots of *A. ischaemum* are slightly higher than the percentages of soluble nitrogen reported by SPRAGUE and SULLIVAN (**10**) for orchard grass but are considerably less than the values obtained by MACVICAR (**9**) for cereals and the annual rye grass, *Lolium multiflorum* Lam. Since the extraction methods of CHIBNALL (**2**) and LUGG (**8**) were different from those used here, no accurate comparisons are possible. The existence of free amino acids in grasses has, however, been well established by both Chibnall and Lugg. The relative abundance of each of the amino acids in a free state is comparable with the abundance as determined after acid hydrolysis of similar plant samples. Certain of the amino acids, particularly histidine and serine, were, however, not detected among the free amino acids; although they occurred in high concentrations in the acid hydrolysates. In view of the relatively important concentrations of amide nitrogen found by CHIBNALL (**2**) in grass and by STEWARD *et al.* (**11**) in alfalfa, it is indeed surprising that such a relatively small amount of asparagine and almost no glutamine were found in the free amino acid fractions. Controlled experiments failed to indicate any significant loss of glutamine as a result of the drying procedure used in this study.

The amino acids detected upon acid hydrolysis of samples of this bluestem agree closely in identity with those found by LUGG (**8**) in orchard grass. Data on tyrosine are, however, doubtful and data on cystine are lacking. Chibnall reports that it is not possible to detect cystine or tryptophan after

acid hydrolysis. He also reports that tyrosine and methionine are partly destroyed by hydrolysis.

The crude protein percentage can be calculated either by multiplying the observed total nitrogen assay results by 6.25 or by totalling the figures of the various plant parts corrected for the relative weights of these parts. Such values for *A. ischaemum* not only agree with each other fairly well but also agree in general range with LUGG's (**8**) values.

The regularity of occurrence of the same amino acids in the different parts of *A. ischaemum* over successive stages of its growth cycle suggests a fundamental protein content which does not change markedly either from one plant organ to another or with time. Variation in the abundance of the amino acids during the growth cycle or following clipping is indicative of changes in the total amount of protein. This finding is in agreement with the earlier observations of LUGG (**8**) and CHIBNALL (**2**).

The failure of clipping to alter the amino acid distribution indicates that no change other than in total protein has been effected by partial defoliation. It is possible that because of the removal of much of the leaf tissue, the protein percentage in both the remaining parts of the plant and in the subsequently formed new leaves is relatively higher. This relative protein content advantage is again lost as leaf area and carbohydrate synthesis ability increase.

Summary

The percentage of total nitrogen in King Ranch bluestem, *Andropogon ischaemum*, was observed to decrease with increase in size of the plants. Those plant parts which had the highest total nitrogen also had the highest percentages of hydrolyzable amino acids.

The free (water-soluble) amino acids and the amino acids detectable after acid hydrolysis of similar plant samples gave evidence of regularity in content. The amino acid content appears not to be altered by maturation of the plant or its parts or by partial defoliation.

Seeds of *Andropogon ischaemum* L. used in this experiment were kindly furnished by the San Antonio Nursery of the Soil Conservation Service of the United States Department of Agriculture. This work is part of a project to which several individuals and corporate groups have lent support. It is a pleasure to acknowledge support from the following: Mr. E. W. Brown, Jr.; Mr. Joe Bridwell; Mr. J. Catto, Jr.; Mr. Bryant Edwards; Mr. Ewing Halsell; Mr. L. B. Hapgood; Mr. Richard King; Mr. Tom Lasater; Mr. M. H. W. Ritchie; Mr. G. R. White; Mrs. Anne Burnett Windfohr; Humble Oil & Refining Company; King Ranch; San Antonio Loan & Trust Company; Sawyer Cattle Company; and Swift & Company.

THE PLANT RESEARCH INSTITUTE
THE UNIVERSITY OF TEXAS
AUSTIN, TEXAS

LITERATURE CITED

1. Bukey, F. S. and Weaver, J. E. Effects of frequent clipping on the underground food reserves of certain prairie grasses. Ecol. **20**: 246–252. 1939.
2. Chibnall, A. C. Protein Metabolism in the Plant. Yale University Press. 1939. 306 pp.
3. Dent, C. E. The Amino Aciduria in Fanconi Syndrome. A study making extensive use of techniques based on paper partition chromatography. Biochem. Jour. **41**: 240–253. 1947.
4. Dent, C. E. A study of the behavior of some sixty amino acids and other ninhydrin-reacting substances on Phenol—"collidine" filter paper chromatograms with notes as to the occurrence of some of them in biological fluids. Biochem. Jour. **43**: 169–180. 1948.
5. Fudge, J. F. and Fraps, G. S. The chemical composition of grasses of Northwest Texas as related to soils and to requirements for range cattle. Texas Agr. Expt. Sta. Bul. 669. 1945. 56 pp.
6. Holscher, Clark E. The effect of clipping bluestem wheatgrass and blue grama at different heights and frequencies. Ecol. **26**: 148–156. 1945.
7. Leukel, W. A., Camp, J. P., and Coleman, J. M. Effect of frequent cutting and nitrate fertilization on the growth behavior and relative composition of pasture grasses. Univ. Florida Agr. Expt. Sta. Bul. 269. 1934. 48 pp.
8. Lugg, J. W. H. The amide, tyrosine and tryptophan contents and the sulphur distributions (cystine plus cysteine and methionine contents) of some plant leaf protein preparations. Biochem. Jour. **32**: 2123–2128. 1838.
9. MacVicar, Robert. The nitrogen composition of cereal grasses. I. Principal nitrogen fractions. Plant Physiol. **24**: 367–372. 1949.
10. Sprague, V. G. and Sullivan, J. T. Reserve carbohydrates in orchard grass clipped periodically. Plant Physiol. **25**: 92–102. 1950.
11. Steward, F. C., Thompson, J. F., Millar, F. K., Thomas, M. D., and Hendricks, R. H. The amino acids of alfalfa as revealed by paper chromatography with special reference to compounds labelled with S^{35}. Plant Physiol. **26**: 123–135. 1951.
12. Stuart, Neil W. The determination of amino nitrogen in plant extracts. Plant Physiol. **10**: 135–148. 1935.
13. Sullivan, J. T. and Sprague, V. G. Compositon of the roots and stubble of perennial rye grass following partial defoliation. Plant Physiol. **18**: 656–670. 1943.
14. Tsao, Tsung-Hsun, Rabideau, G. S., and Whaley, W. G. The phosphorus uptake of *Andropogon ischaemum* L. at various stages of development. Plant Physiol. **25**: 653–665. 1950.
15. Williams, R. J. and Kirby, Helen. Paper chromatography using capillary ascent. Science **107**: 481–483. 1948.

OCCURRENCE OF *BACILLUS CEREUS* IN MARYLAND SOILS WITH FRENCHED TOBACCO

ROBERT A. STEINBERG

Received April 6, 1951

Frenching of tobacco is a non-infectious, or physiological, disease characterized by reticular chlorosis of the leaves, cessation of stem and branch elongation, strap-leaf formation, and greatly increased leaf number. These symptoms develop usually in the order given. The roots appear to be normal except for slight stunting and thickening. Recovery may occur spontaneously or when the plant is transplanted to a normal soil.

Natural isoleucine can be used to duplicate these symptoms in aseptic culture, water culture, and soil (**8**). Necessary concentrations range from about 10 p.p.m. up, depending on the type of culture and variety of tobacco. Frenching in the field does not seem to be associated with the presence of isoleucine in soil (**11**). The accumulation of free isoleucine in the tobacco plant and the hormone-like morphological responses caused thereby appear, therefore, to result from functioning of an internal receptor mechanism only. A toxin (**7**) or another organic substance, presumably sometimes present in the soil because of bacterial activities, may be assumed to be the primary cause. Bacteriological studies afford some evidence that a noxious substance may be produced as a result of microbial activities in the soil. A survey of some 60 common species or strains of soil bacteria has revealed that in pure culture, only one was clearly capable of reproducing the symptoms of frenching (**9, 10**). This was *Bacillus cereus* Frankland & Frankland.

A hypothesis of a microbiologically produced substance as a cause of frenching appears to be a reasonable one in view of some other observations. Responses of frenched plants to minerals (**1, 6, 7, 12**), to rainfall (**1**), to soil acidity (**5, 12**) and to temperature (**4**) are known to be erratic. It has also been observed that frenched plants in the greenhouse may recover spontaneously, without necessarily any loss in the frenching capacity of the soil. When frenched plants that had eventually begun forming normal shoots were replaced with normal seedlings, these latter subsequently frenched severely. Disturbances in the biological equilibria in the soil or between the plant and the soil that enable the saprophytic *B. cereus* to develop excessively could well lead to the more or less transitory appearance of the hormone-like symptoms of frenching. Factors involved in a relationship of this nature are entirely different than those involved in classical parasitism.

It seems probable that if frenching is due to a toxin secreted by *Bacillus cereus*, the population counts of this organism in the tobacco plant rhizosphere should be correlated with occurrence of frenching. There is little

information about the number and types of Bacillus normally present on plant roots (**3**). It is known that bacteria in general are especially numerous on root surfaces (**2**), *i.e.*, rhizoplane.

Methods

During the summer of 1950 and in each of seven fields, separate samples of soil were taken from about the roots of apparently normal and severely frenched tobacco plants. Similar separate samples were taken of the roots with most of the soil shaken off. Comparative samples of normal and frenched plants were always from locations as closely adjacent as possible, sometimes within three feet, and from plants of the same size. Usually small plants (late plantings) were used in order to avoid destroying plants with many well-formed leaves. In two instances (the Wilson and Rawlings farms), the normal plants did not appear to be entirely free of all symptoms of frenching but were the best that could be obtained.

After collection, portions of the soil samples showing frenching of tobacco in the field were immediately replanted, under greenhouse conditions, with normal seedlings. Soil acidities were determined at the same time with a glass electrode apparatus. Frenching responses with each soil were observed with three successively transplanted seedlings over a period of many months. These tests were primarily for the purpose of determining the actual status of the soils when sampled, since greenhouse tests would indicate existence of a two-week lag between frenching stimulus and appearance of symptoms. A similar or greater lag presumably exists between disappearance of stimulus and appearance of normal growth. While it may be assumed that a difference should be present when averages for normal and abnormal are compared on the basis of plant responses in the field, there is a definite degree of uncertainty regarding the status of the individual samples at time of collection. This uncertainty is eliminated to some extent by grouping of samples on the basis of greenhouse tests for soil abnormalities in retentiveness. The soil samples showing very high retentiveness were presumably still abnormal at time of collection, whereas those showing no retentiveness had presumably become normal again even though the plants were as yet severely frenched.

Results

To facilitate examination, the data of table I have been arranged on the basis of degree of retention of frenching stimulus by the seven presumably abnormal soils. While average population of *B. cereus* for apparently normal soils (matched controls) was about 76 thousand, that for abnormal soils was about 126 thousand, or 65% more. The average counts for the roots growing in these soils were 10 million for normal plants and around 30 million for frenched plants, an increase of 200%.

Six of the matched pairs of soils showed a greater bacterial population with frenching, while one pair had identical counts. However, only four of these matched pairs gave higher root counts with frenched plants, though

all counts were much higher than for soils. This discrepancy vanishes if comparison is made on the basis of average values with roots for responses in greenhouse trials. Those soils possessing high retentiveness gave very high root counts of *B. cereus* in frenched as compared with normal soils. They diminished sharply with decreased retentiveness from 75 to 6 million. The frenched/normal ratios decreased from 12 to 0.2. The corresponding values for the three groups of soils do not show the same regularity. This is not unexpected since these are actually spore and not total population counts, and the ratios of the two may vary. Moreover, bacteria in the soil at relatively greater distances from the roots would be expected to have relatively less influence than those much closer.

TABLE I

B. cereus POPULATIONS FOUND WITH NORMAL AND FRENCHING SOILS AND ROOTS AND THEIR RELATION TO SOIL RETENTIVENESS OF FRENCHING CAPACITY IN THE GREENHOUSE

Source of materials	Soil pH	Retention of frenching capacity by soil	*B. cereus* populations of field samples					
			Thousands per gram of rhizosphere soil			Millions per gram of oven-dried roots		
			Normal	Frenched	Frenched / normal	Normal	Frenched	Frenched / normal
Hamilton Farm	6.83	High	20	90		0.198	78.98	
Rawlings Farm	6.80		135	145		12.37	72.14	
Average	6.82		77.5	117.5	1.52	6.23	75.56	12.02
Wilson Farm	6.38	Low	95	95		2.45	19.35	
Conner Farm	6.13		85	125		1.28	30.08	
Addison Farm*	6.08		65	85		2.48	0.74	
Average	6.20		81.7	101.7	1.24	2.07	16.71	8.08
Bowen Farm	5.78	None	60	220		17.08	4.02	
Tucker Farm	6.49		75	125		34.58	8.43	
Average	6.09		65.5	172.5	2.56	25.83	6.05	0.23
Total Average	6.21		76.43	126.4	1.65	10.06	30.53	3.03

* Frenching slight and transient with first greenhouse planting; none on second planting; and slight on third planting.

While there seems to be an indication of an inverse trend between soil acidities and retention of frenching capacity, the variations are too small to permit a definite interpretation. Acidity values for severe frenching during the previous year had ranged from pH 5.63 to 6.91. The average for four highly retentive soils was pH 6.43, for a slightly retentive soil, 6.91; and for one of zero retentiveness it was 5.63.

Discussion

The observation (**9, 10**) that diffusates from *Bacillus cereus* caused frenching of tobacco in aseptic culture suggested that this bacterial species might bear some relationship to the disease under field conditions. The

data currently accumulated support this assumption to the extent that higher populations of *B. cereus* have been encountered in the rhizosphere of frenched tobacco than in the rhizosphere of normal tobacco. Furthermore, some exceedingly high populations of *B. cereus* have been found associated with the roots of frenched tobacco. The *B. cereus* counts of 72 and 78 millions (table I) for roots of frenched tobacco are several times greater than the Bacillus counts hitherto reported for the roots of healthy plants grown under greenhouse conditions (**3**). They are also greater than the *B. cereus* counts for roots of apparently normal tobacco plants growing in the same fields. Maximum weight should be given root counts in interpretation of these data since proximity is, without doubt, an important factor in stimulation.

The fact that a large population of a particular soil microörganism is found associated with the roots of frenched tobacco is not in itself clear evidence of a causal relationship of that organism to the disease. Rhisosphere and rhizoplane populations are known to vary greatly with differ physiological conditions of the plants themselves, and it is entirely possible that the roots of frenched tobacco are particularly favorable for colonization by *B. cereus*. In that case, *B. cereus* would bear no causal relationship to the disease. Nevertheless, the observation that diffusates of *B. cereus* have been found capable of producing the frenching disease while those from about 60 other common soil bacteria have failed to do so does emphasize the possible significance of the present data.

Summary

Samples of soils growing normal and severely frenched tobacco and of normal and frenched roots were collected on seven different southern Maryland farms during the summer of 1950, and the populations of *Bacillus cereus* Fr. & Fr. were determined.

In six of seven paired samples of rhizosphere soil, higher populations of *B. cereus* were found for the frenching soil than were found in normal soils. The average *B. cereus* population of the frenching soils was 1.65 times that of normal soils. Roots of frenched tobacco showed especially large populations of *B. cereus*, with values several times those hitherto reported for normal plant roots. The probability that *B. cereus* bears some causal relationship to frenching of tobacco is discussed.

The writer is indebted to Dr. Francis E. Clark of this Bureau for determining bacterial counts and for suggestions concerning the preparation of the manuscript; and to Robert Hall, Extension Agent for Calvert County, Maryland, for his aid in locating and collecting samples.

BUREAU OF PLANT INDUSTRY
BELTSVILLE, MARYLAND

LITERATURE CITED

1. BUCHI, H. Beitrag zum Problem des Frenching. Mitt. des Forschungs- und Beratungstell der Sota., Pflanzenphysiol. Inst. E.T.H., pp. 1–15. 1946.
2. CLARK, FRANCIS E. Soil microörganisms and plant roots. Adv. Agronomy **1**: 241–288. 1949.
3. CLARK, FRANCIS E. and SMITH, D. H. Occurrence of spore-forming bacteria on plant roots and in soil. Proc. Soil Sci. Soc. Amer. **14**: 199-202. 1950.
4. JONES, LINUS H. Soil temperature as a factor in the frenching of tobacco (*Nicotiana tabacum* L.). Plant Physiol. **23**: 560–575. 1948.
5. KARRAKER, P. E. and VALLEAU, W. D. Frenching of tobacco and liming. Jour. Amer. Soc. Agron. **22**: 283–285. 1930.
6. SHEAR, O. N. Field and laboratory studies on frenching of tobacco. Virginia Agr. Expt. Sta. Res. Bull. **49**, pp. 1–14. 1933.
7. SPENCER, ERNEST L. Studies on frenching of tobacco. Phytopath. **25**: 1067–1084. 1935.
8. STEINBERG, ROBERT A. Growth responses of tobacco seedlings in aseptic culture to diffusates of some common soil bacteria. Jour. Agr. Res. **75**: 199–206. 1947.
9. STEINBERG, ROBERT A. Symptoms of amino acid action on tobacco seedlings in aseptic culture. Jour. Agr. Res. **78**: 733–741. 1949.
10. STEINBERG, ROBERT A. The relation of certain soil bacteria to frenching symptoms of tobacco. Bull. Torr. Bot. Club **77**: 38–44. 1950.
11. STEINBERG, ROBERT A., BOWLING, JOHN D., and MCMURTREY, JAMES E. Accumulation of free amino acids as a chemical basis for morphological symptoms in tobacco manifesting frenching and mineral deficiency symptoms. Plant Physiol. **25**: 279–288. 1950.
12. VALLEAU, W. D. and JOHNSON, E. M. Tobacco frenching—a nitrogen deficiency disease. Kentucky Agr. Expt. Sta. Res. Bull. **281**: 179–253. 1927.

FURTHER EVIDENCE FOR A CHEMICAL REACTION BETWEEN PLANT GROWTH-REGULATORS AND A PLANT SUBSTRATE

CORWIN HANSCH, ROBERT M. MUIR AND ROBERT L. METZENBERG, JR.

Received April 17, 1951

Introduction

The substitution of atoms or groups in both ortho positions of phenoxyacetic, phenylacetic, phenylbutyric and indoleacetic acids causes the compounds to be ineffective in promoting the elongation of sections of Avena coleoptiles (**9, 15**). The obvious interpretation is that these atoms or groups in the ortho position block a chemical reaction taking place at that point. Considerable evidence exists that electronegative atoms or groups attached to the benzene ring in phenoxyacetic and phenylacetic acids enhance the activity of these compounds, and this feature suggests that the substrate reacting at the ortho position is nucleophilic in nature (**15**). BENTLEY (**2**), however, has reported that 2,3,6-trichlorobenzoic acid is very effective in promoting the elongation of coleoptile sections, although both ortho positions are occupied by chlorine atoms. Investigations of the activities of a number of benzoic acid derivatives have shown that the active compounds have one or both ortho positions occupied by electronegative atoms or groups (**9, 26**), and it has been suggested that with these compounds the reaction takes place by a displacement of the electronegative atom or group by the nucleophilic substrate (**9**).

One of the active derivatives of benzoic acid is 2,6-dichlorobenzoic acid. If such a substance does react at an ortho position by nucleophilic displacement of a chloride ion, then chemical analysis of the tissue and the solution in which growth has taken place should detect an increase in the amount of chloride ion. Such analyses were made and an increase in Cl^- was detected.

Materials and methods

Avena plants were grown and coleoptile sections were harvested as reported previously (**16**) except that the sand in which the plants were grown was freed of chloride by heating at 150° F overnight with concentrated sulphuric and nitric acids. The sand was then washed with hot, doubly distilled water until the washings gave a negative sulphate test with barium chloride. All apparatus used in the experiment was cleaned scrupulously and then washed with doubly distilled water. Best results were obtained when all procedures were conducted in a small, carefully scrubbed, dark room in which only the investigator worked.

One lot of sections was placed in 25 ml. of the solution of the chemical, and a second lot was placed in 25 ml. water to serve as a control. The sections were allowed to grow for eight hours before analysis. This period of time for growth was used to avoid bacterial activity. After eight hours, the sections were removed; and the liquids were evaporated to dryness under a heat lamp. The chloride content of the residues was determined along with that of the sections. The sections were ground thoroughly in a mortar and dried until crisp under a heat lamp to insure rupture of all cells. The dried sections were ground again and extracted six times with hot water, the water being decanted into a centrifuge tube after each extraction. The combined washings were centrifuged to remove suspended material and evaporated to dryness under the heat lamp.

The analysis for chloride was made by the procedure developed by KIRK and coworkers (**12**) for the determination of chloride in the presence of protoplasmic material. Actually very little such material was present in the solutions analyzed since the protein material was rendered insoluble by the heat treatment and removed by centrifugation. Titrations were made with 0.01 *N* $AgNO_3$, and the volume of the solution at the beginning of the titration was 0.8 ml.

Experimental results and discussion

The validity of the technique employed was established by several preliminary experiments. Comparable data were obtained from five experiments and the data from two such experiments are summarized in table I. The data show that the amount of chloride ion does increase when growth takes place in the presence of 2,6-dichlorobenzoic acid or 2,4-dichlorobenzoic acid. The possibility that the increase of chloride ion is due to hydrolysis of the compounds under the conditions of the experiment was examined by evaporation of their solutions under the heat lamp and also by boiling

TABLE I

CHLORIDE CONTENT OF COLEOPTILE SECTIONS AND GROWTH MEDIUM AFTER GROWTH PERIOD OF EIGHT HOURS

Exp. no.	Compound	Molar conc.	No. of sections	Length of sections	Fresh wt. of sections	Micrograms of chloride	
						Sections and solution	Sections and water control
				mm.	*g.*		
1.	2,6-dichloro-benzoic acid	5×10^{-4}	50	5.5	0.35	46	38
2.	2,6-dichloro-benzoic acid	1×10^{-4}	162	3.0	0.62	75	50
	2,4-dichloro-benzoic acid	1×10^{-4}	162	3.0	0.62	62	50

the compounds in aqueous solution. No ionic chloride was obtained in either treatment of 2,6-dichlorobenzoic acid. The evaporation of 25 ml. of 1×10^{-4} M 2,4-dichlorobenzoic acid solution under the heat lamp resulted in the detection of 2.5 micrograms of ionic chloride. This observation indicates that Cl^- is more easily removed from the 2,4-acid. However, during the growth of the sections much more chloride is released from the 2,6-acid. The 2,4-acid does not promote elongation of the sections, and the chloride released during the growth period (12 micrograms) may be the result of hydrolysis. The possibility also exists that the chloride may be removed from the 4-position rather than the 2-position.

Quantitative data on the ease of removal of Cl from the benzoic acids are not available in the chemical literature; however, such data are available for the analogous chloronitrobenzenes. Nucleophilic groups remove chlorine 144 times faster from the 2,4-dichloronitrobenzene than from the 2,6-dichloronitrobenzene and both chlorine atoms are removed (**1**). Presumably the greater stability of the 2,6-dichloronitrobenzene toward nucleophilic displacement is due to the steric inhibition of resonance (**1**) which

TABLE II

PERCENTAGE ELONGATION OF SECTIONS OF COLEOPTILES IN SOLUTIONS OF INDOLEACETIC ACIDS AS COMPARED WITH PERCENTAGE ELONGATION IN WATER

	Molar concentrations $\times 10^{-4}$							
	2.0	1.0	0.5	0.1	0.05	0.01	0.005	0.001
4-Chloroindoleacetic acid	+7	+20	+20	+19	+28	+21	+17	+13
Indoleacetic acid				+24		+20		+7

results from the substitution of the two ortho positions forcing the nitro group out of the plane of the ring. The same steric hindrance of resonance has been shown (**17**) to occur with 2,6-dichlorobenzoic acid.

The larger amount of chloride released from 2,6-dichlorobenzoic acid in experiment 2 was correlated with greater elongation of the sections in the lower concentration of the chemical. At concentrations greater than 2×10^{-4} M the promoting effect of the compound on elongation decreases (**16**). If one assumes that every chloride ion displaced represents a molecule of 2,6-dichlorobenzoic acid reacting, the data of experiment 2 show that 1.13×10^{-6} mole of the compound reacts per gram of coleoptile tissue and only 28% of the molecules present actually are involved in the reaction.

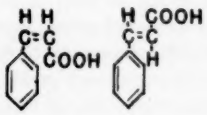
These data are additional evidence for the hypothesis that compounds which promote elongation of the cells do so by a chemical reaction at the ortho position involving a displacement of hydrogen (phenoxyacetic, phenylacetic, and phenylbutyric acids) or electronegative atoms or groups (benzoic acids). Evidence exists that the ortho effect is operative for indoleacetic acids (**9, 16**). Substitution at the 2 position diminishes the activity of in-

doleacetic acid; substitution at the 2 and 4 positions essentially nullifies the activity. It was of interest therefore to determine the activity of a compound substituted only at the 4 position, and to this end the synthesis of 4-chloroindoleacetic acid was carried out and its activity is shown in table II.

It is apparent from the data in table II that the substituted acid has an effect on elongation which is equal to or greater than the effect of indoleacetic acid. This observation may be explained by the aromatic nature of the five-membered ring (**14**) and the favorable electronic effect of substitution reactions in this ring brought about by the chlorine at position 4.

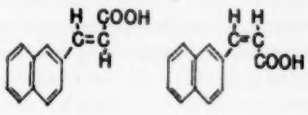
VELDSTRA (**23**) has presented results of investigations on the growth activity of cis and trans acids such as cinnamic acid and the phenylcyclopropane carboxylic acids which do not seem to be accounted for by the hypothesis of the ortho effect. In the following acids, he reports the cis isomers are active and the trans isomers are not (evidence for the structures of the naphthylacrylic acids and the tetra hydronaphthylideneacetic acids has been obtained by HAVINGA and NIVARD, **10**):

Cinnamic acids



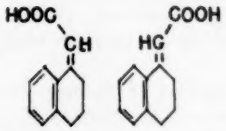
Cis Trans

B-Naphthyl-1-acrylic acids



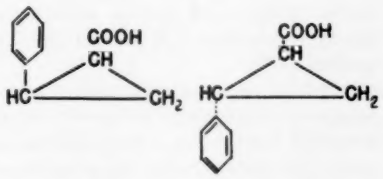
Trans Cis

1,2,3,4-Tetrahydronaphthylidene acetic acids



Cis Trans

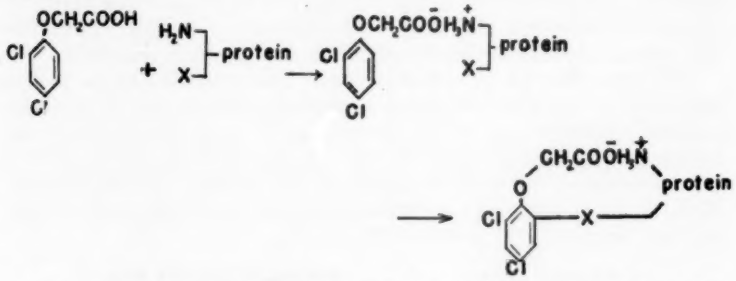
1-Phenylcyclopropane carboxylic acids



Cis Trans

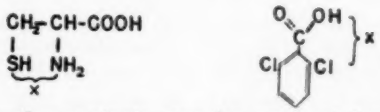
The activity of the cis isomer is explained by Veldstra by postulating that in these isomers the carboxyl groups are out of the plane of the ring while in the trans isomers the carboxyl groups are in the plane of the ring, and the carboxyl group must be out of the plane of the ring for activity (**4, 24**). However, with certain assumptions the activity of the cis isomers can be interpreted in terms of a reaction at the ortho position. Since a carboxyl group or a group convertible to a carboxyl group is essential for growth activity, a reasonable assumption is that the function of this group is to

attach the molecule to a protein by reaction with a basic group such as an amine. Such a reaction would be fast compared to the nucleophilic substitution at the ortho position. The first step would be salt formation. Following this attachment, reaction at the ortho position with another point on the protein would lead to a cyclic reaction product according to the following equation:



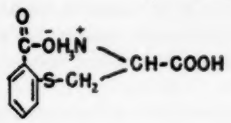
The ring could form with a minimum of strain in the cis form of the acids. In the tetrahydronaphthylideneacetic acid the carboxyl group is cis to a ring in each form; however, the reaction could take place only through the unsaturated ring (**13**). In the trans forms of the acids much greater strain (presumably prohibitive) would be involved in the formation of such a ring. Using Hirschfelder models, a ring can be formed from cis cinnamic acid and cysteine by having an amide linkage between the cinnamic acid carboxyl group and the cysteine amine group with the sulphur of the cysteine attached at the ortho position on the benzene ring. It is not possible to form such a structure using the trans form of cinnamic acid. Using a salt linkage instead of an amide linkage would involve less strain however. Strains of the type described will prevent the formation of certain rings in organic molecules (**7**).

Cysteine or a cysteinyl unit of a protein is regarded as the most likely substrate reacting with the growth regulator because of the distince, X, between the basic group, $—NH_2$, and the nucleophilic group, —SH. The minimum distance separating the groups would be that in the benzoic acid structure since some derivatives of the acid have considerable effect in causing elongation. This minimum distance is equal to X as is shown in the following formulae:

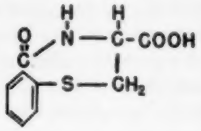


The cyclic reaction products may be constructed with Hirschfelder models for the combination of cysteine and benzoic or phenoxyacetic acids using either an amide or a salt linkage as shown below:

Benzoic acids

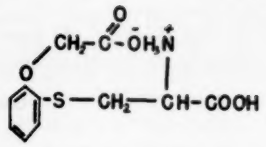


Salt linkage

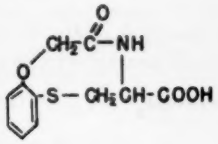


Amide linkage

Phenoxyacetic acids



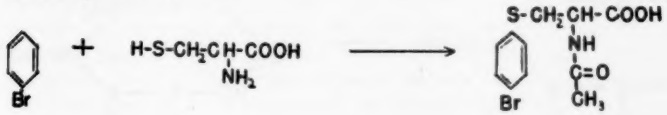
Salt linkage

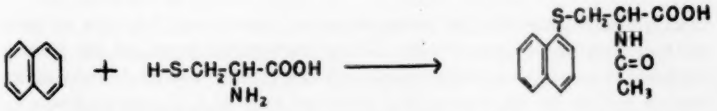


Amide linkage

The rings would have a minimum of seven members in the case of the amide linkage of the benzoic acids, and as many as 11 members in the case of the γ-phenylbutyric acid with the salt linkage.

The nucleophilic substitution of an aromatic nucleus is not unreasonable from either the chemical or biological point of view. A cysteine unit is introduced into aromatic molecules in various animals (**5**, **11**, **18**) in the following manner:





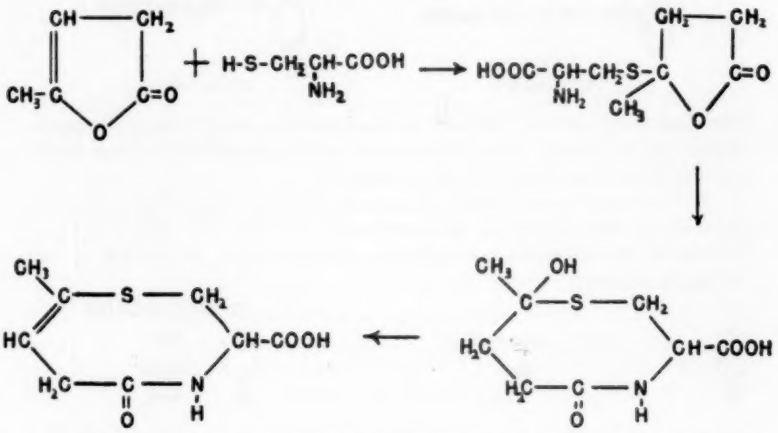
It is interesting to note that the amino group is acetylated by the animal which would correspond to the amide formation as postulated for the growth regulators. STEKOL (**19**) has shown that naphthalene or bromobenzene stop growth in animals, but the effect of these two compounds can be reversed by feeding L-cysteine or DL-methionine. These observations indicate the importance of the —S—S— ⇌ —SH equilibrium in animal growth.

The observation of THIMANN and BONNER (**21**, **22**) that iodoacetate, arsenite and *p*-chloromercuribenzoate (which react with —SH groups) inhibit the elongation of Avena and Pisum sections may be interpreted as indicating a combination of the growth regulators with sulfhydryl enzymes.

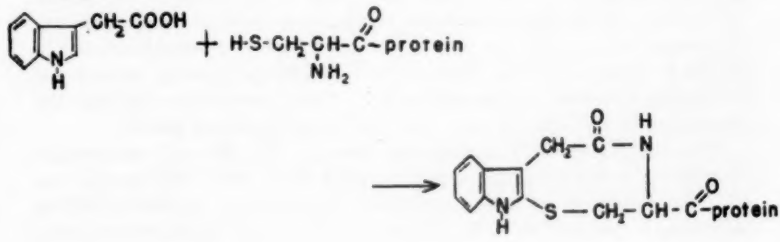
The inhibition of growth could not be reversed with 2,3-dithioglycerol, butane-dithiol-2,3 or thioglycolate (**22**). If the growth regulators react with —SH groups and the inhibitors react more rapidly with the —SH groups or an excess of sulfhydryl compounds is added, a reversal of inhibition would not be possible.

BONNER (**3**) has shown that L-cysteine inhibits the elongation of Avena coleoptile sections, and this may be explained by the reaction of the cysteine with the growth hormone thus preventing the hormone from reacting with a cysteinyl unit of a protein molecule.

A variety of unsaturated lactones of the general structure inhibit growth of plants (**8, 25**). CAVALLITO and HASKELL (**6**) have shown that these lactones react with cysteine according to the following mechanism:



Thus the inhibiting effect of the lactones on growth could be due to their reaction with the cysteinyl unit of a protein which prevents the growth regulator from reacting at that point. If the growth regulators activate an enzyme system by reaction with a cysteinyl part of a protein molecule as follows:



then an unsaturated lactone, iodoacetate, arsenite or any substance which would effectively compete with the growth regulator for the cysteinyl enzyme unit would inhibit growth provided the cyclic reaction product would not operate in the next step of the growth reaction.

Another interesting aspect of the relationship of chemical structure and physiological activity in the growth regulators which may be explained on the basis of the ortho reaction is the difference in the activity of D and L optical isomers. The data on the isomers has been summarized recently by THIMANN (**20**). As an example, the D isomer of 2,4-dichloro-α-naphthoxypropionic acid is twice as active as the racemic mixture. If optically active growth regulators react with an optically active amino acid such as cysteine in a protein enzyme, then the resulting diastereoisomers differ greatly in their chemical and physical properties. Thus, the two enzymes resulting from the reaction of the D and L forms of the growth regulator with the protein would have different properties and one might be more effective in the growth reaction than the other.

Summary

Increased elongation of Avena coleoptile sections brought about by 2,6-dichlorobenzoic acid is accompanied by the release of chloride ion. This observation substantiates the interpretation of the reaction of the growth regulator as a displacement of the electronegative atom by a nucleophilic substrate.

The reaction of the growth regulator through the carboxyl group and the ortho position requires a basic group and a nucleophilic group on the substrate which are separated by a minimum distance corresponding to that between the carboxyl group and the ortho position of benzoic acid. The cysteinyl unit of a protein appears to be the most likely such substrate in the plant cell. Cyclic reaction products between cysteine and the benzoic and phenoxyacetic acids are possible on chemical grounds.

The different activities of D and L optical isomers and cis and trans isomers, and the inhibitory effects of unsaturated lactones, arsenite, iodoacetate, *p*-chloromercuribenzoate, and cysteine may be explained by the ortho reaction hypothesis for plant growth-regulators.

CHEMISTRY DEPARTMENT
POMONA COLLEGE
CLAREMONT, CALIFORNIA
AND
BOTANY DEPARTMENT
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA

LITERATURE CITED

1. ALEXANDER, E. R. Ionic Organic Reactions. p. 21. Wiley, New York. 1950.
2. BENTLEY, J. A. Growth-regulating effect of certain organic compounds. Nature **165**: 449–450. 1950.

3. Bonner, J. Limiting factors and growth inhibitors in the growth of the Avena coleoptile. Amer. Jour. Bot. **36**: 323–332. 1949.
4. Booij, H. L. and Veldstra, H. Researches on plant growth regulators. XVI. The effect of plant growth substances on coacervates. Biochim. Biophys. Acta. **3**: 260–277. 1949.
5. Bourne, M. C. and Young, L. The metabolism of naphthalene in rabbits. Biochem. Jour. **28**: 803–808. 1934.
6. Cavallito, C. J. and Haskell, T. H. The mechanism of action of antibiotics. The reaction of unsaturated lactones with cysteine and related compounds. Jour. Amer. Chem. Soc. **67**: 1991–1994. 1945.
7. Gilman, H. Organic Chemistry. 2nd Ed. pp. 330–336, 447–449, 480. Wiley, New York. 1943.
8. Goodwin, R. H. and Taves, C. The effect of coumarin derivatives on the growth of Avena roots. Amer. Jour. Bot. **37**: 224–231. 1950.
9. Hansch, C. and Muir, R. M. The ortho effect in plant growth-regulators. Plant Physiol. **25**: 389–393. 1950.
10. Havinga, H. and Nivard, R. J. Ultra violet absorption spectra and stereochemical structure of plant growth substances of the cis cinnamic acid type and of stilbestrol. Rec. trav. chim. Pays-Bas. **67**: 846–854. 1948.
11. Ing, H. R., Bourne, M. C., and Young, L. CXIII. Synthesis of 1-α-naphthylmercapturic acid. Biochem. Jour. **28**: 809–810. 1934.
12. Kirk, P. L. Quantitative Ultramicroanalysis. Wiley, New York. 1950.
13. Koepfli, J. B., Thimann, K. V., and Went, F. W. Phytohormones: Structure and physiological activity. I. Jour. Biol. Chem. **122**: 763–780. 1937–1938.
14. Morton, A. A. The Chemistry of Heterocyclic Compounds. pp. 63–65. McGraw-Hill, New York. 1946.
15. Muir, R. M. and Hansch, C. The relationship of structure and plant-growth activity of substituted benzoic and phenoxyacetic acids. Plant Physiol. **26**: 369–374. 1951.
16. Muir, R. M., Hansch, C., and Gallup, A. H. Growth regulation by organic compounds. Plant Physiol. **24**: 359–366. 1949.
17. Ross, S. D. Steric inhibition of resonance in chlorinated benzoic acids. Jour. Amer. Chem. Soc. **70**: 4039–4041. 1948.
18. Stekol, J. A. The determination of p-bromophenylmercapturic acid in the urine of a dog. Jour. Biol. Chem. **113**: 279–287. 1936.
19. Stekol, J. A. Studies on the mercapturic acid synthesis in animals. V. The effect of naphthalene on the growth of rats as related to diets of varying sulphur content. Jour. Biol. Chem. **121**: 87–98. 1937.
20. Thimann, K. V. The synthetic auxins: Relation between structure and activity. In Plant Growth Substances, F. Skoog, Ed. pp. 21–36. University of Wisconsin Press, Madison. 1951.

21. THIMANN, K. V. and BONNER, W. D. Experiments on the growth and inhibition of isolated plant parts. I. The action of iodoacetate and organic acids on the Avena cole[illegible] Amer. Jour. Bot. **35**: 271–281. 1948.
22. THIMANN, K. V. and BONNER, W. D. Exp[illegible]ents on the growth and inhibition of isolated plant parts. II. The action of several enzyme inhibitors on the growth of the Avena coleoptile and on Pisum internodes. Amer. Jour. Bot. **36**: 214–221. 1941.
23. VELDSTRA, H. Some chemical aspects of growth substance action. Reported at the Seventh International Botanical Congress. Stockholm. 1950.
24. VELDSTRA, H. and BOOIJ, H. L. Researches on plant growth regulators. XVII. Structure and activity. On the mechanism of action. III. Biochim. Biophys. Acta. **3**: 278–312. 1949.
25. VELDSTRA, H. and HAVINGA, E. On the physiological activity of unsaturated lactones. Enzymologia **11**: 373–380. 1945.
26. WEINTRAUB, R. L. and NORMAN, A. G. Plant growth-regulators. Econ. Bot. **3**: 289–298. 1949.

THE INFLUENCE OF VARIOUS SODIUM AND POTASSIUM SALTS UPON THE GROWTH OF YOUNG AVENA SEEDLINGS[1]

BRUCE J. COOIL

Received March 19, 1951

A relationship between the four-carbon acids and the growth of oat seedlings was reported by ALBAUM and COMMONER (**1**), who found that when intact seedlings were grown in dilute solutions of iodoacetate, the growth of the coleoptiles was inhibited. The addition of fumarate to the solutions resulted in what appeared to be a reversal of this growth inhibition. The results of these authors and those obtained by COMMONER and THIMANN (**4**) using isolated coleoptile sections were taken to indicate operation of a four-carbon acid cycle participating directly in the growth processes. ALBAUM and EICHEL (**2**) later showed increases in coleoptile length to result when intact oat seedlings were supplied with pyruvate, malate, succinate, fumarate, malonate, or maleate. These responses were obtained in both the presence and absence of iodoacetate. The same acids were also reported to stimulate respiration. The stimulating effects of malonate and maleate could not be reconciled with the view that the various organic acids exerted their influence by operation through a scheme of the type proposed by SZENT-GYÖRGI (**9**), since malonate and maleate are known inhibitors of the system.

Using isolated coleoptile sections, THIMANN and SCHNEIDER (**11**) showed a large response to KCl. In the present study of intact oat seedlings a number of preliminary experiments showed an early growth response to potassium. The response was evident when plants were supplied with sodium or potassium salts in single salt solutions, and also when comparison was made between plants growing in a balanced nutrient solution and in nutrient solutions lacking only potassium. In this connection it was noted that in the experiments of Albaum and Commoner and Albaum and Eichel KOH was used to neutralize the organic acids employed. Accordingly, experiments were conducted to determine whether the results of these authors were indeed responses to organic acids or responses to potassium.

Material and methods

Seeds of *Avena sativa* L. var. Black Norway were soaked in distilled water for about one hour. The glumes were removed during the soaking period. The seeds were germinated in darkness at 26 to 27° C for 24 hours. The culture procedure was then essentially that described by KAISER and

[1] Published by the permission of the Director of the Hawaii Agricultural Experiment Station as Technical Paper no. 225.

ALBAUM (**5**). The germinated seeds were placed in 400 ml. beakers lined with moist filter paper and containing 100 ml. of solution. The seedlings were grown at the same temperature for 140 to 144 hours (*i.e.*, to an age of 164 to 168 hours from the time of soaking). The seedlings were in darkness except during transfer to the beakers and for brief intervals when measurements were being made. These manipulations were made in orange neon light having spectral bands only at wave lengths above 5900 Å. At the end of the growth period the plants were removed from the beakers and determinations were made of coleoptile length, length of the first leaf, length of the longest root and in most cases total root length.

The fumaric, malic, succinic, and malonic acids (all Eastman's) were neutralized to pH 6.0 with NaOH or KOH depending upon which cation was desired. Sodium pyruvate was prepared from Eastman's pyruvic acid by the procedure of ROBERTSON (**7**). Potassium pyruvate was prepared in the same manner as suggested by UMBREIT *et al.* (**12**). The acetates, chlorides, sulphates and nitrates of sodium and potassium were C.P. salts. After final dilution all solutions were tested for reaction and when necessary adjusted to pH 6.0 with NaOH. In those treatments where iodoacetic acid (Eastman's) was used, it was neutralized in the final adjustment of the pH value. Distilled water was used for all solutions. The results reported represent mean values of two to four randomized replications, each replicate consisting of 12 plants in one beaker. Analysis of variance (**8**) was applied to the data of each experiment.

Experimental results

THE INFLUENCE OF SODIUM AND POTASSIUM SALTS UPON GROWTH OF THE COLEOPTILE

In table I are shown the lengths of coleoptiles of Avena seedlings grown in solutions of several sodium and potassium salts in the presence and absence of 2×10^{-5} M iodoacetate. In each experiment iodoacetate alone resulted in a significant reduction in growth of the coleoptile. Each of the potassium salts employed resulted in highly significant growth response both in the presence and absence of iodoacetate. Sodium salts of organic and inorganic acids did not produce increases in coleoptile length comparable to increases resulting from corresponding potassium salts. In the absence of iodoacetate, there was a statistically significant difference between plants grown in water and those grown in 0.005 M sodium malonate. However, similar significant responses resulted from culture in 0.002 M sodium chloride, 0.001 M sodium sulphate and 0.01 M sodium nitrate. In the presence of iodoacetate, significant responses in coleoptile growth were obtained in 0.005 M sodium fumarate, 0.001 M and 0.005 M sodium pyruvate and 0.005 M sodium malate, but responses of equal or superior significance resulted in 0.005 M sodium malonate, 0.001 M and 0.005 M sodium sulphate, 0.001 M and 0.005 M sodium acetate and 0.01 M sodium nitrate.

It is, then, clearly established that Avena seedlings grown in solutions

TABLE I

COLEOPTILE GROWTH RESPONSES RESULTING FROM CULTURE OF SEEDLINGS OF *Avena sativa*, VAR. BLACK NORWAY IN VARIOUS SOLUTIONS.

Experiment no.	No. of replicates	Solution	Without iodoacetate			With iodoacetate 2×10^{-5} M			Iodoacetate inhibition	Least significant difference	
			Length	Increase over control	Response to K	Length	Increase over control	Response to K			
			cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*0.05*	*0.01*
1	4	Water control	4.84	....	....	4.20	....	....	0.64	0.39	0.52
		Sodium fumarate 0.001 M	5.09	0.25	....	4.45	0.25	....	0.64		
		" " 0.005 M	4.84	0.00	....	4.66	0.46	....	0.18		
		Potassium fumarate 0.001 M	5.72	0.88	0.63	5.10	0.90	0.65	0.62		
		" " 0.005 M	5.70	0.86	0.86	5.57	1.37	0.91	0.13		
		Sodium chloride 0.002 M	5.27	0.43	....	4.28	0.08	....	0.99		
		" " 0.010 M	5.04	0.20	....	4.34	0.14	....	0.70		
		Potassium chloride 0.002 M	5.97	1.13	0.70	4.76	0.56	0.48	1.21		
		" " 0.010 M	6.11	1.27	1.07	5.39	1.19	1.05	0.72		
2	3	Water control	4.71	....	....	3.76	....	....	0.95	0.20	0.27
		Sodium malonate 0.001 M	4.75	0.04	....	3.91	0.15	....	0.84		
		" " 0.005 M	4.92	0.21	....	4.22	0.46	....	0.70		
		Potassium malonate 0.001 M	5.39	0.68	0.64	4.67	0.91	0.76	0.72		
		" " 0.005 M	5.32	0.61	0.40	5.13	1.37	0.91	0.19		
		Sodium sulphate 0.001 M	4.92	0.21	....	4.18	0.42	....	0.74		
		" " 0.005 M	4.77	0.06	....	4.51	0.75	....	0.26		
		Potassium sulphate 0.001 M	5.42	0.71	0.50	4.60	0.84	0.42	0.82		
		" " 0.005 M	5.21	0.50	0.44	4.90	1.14	0.39	0.31		

TABLE I *(continued)*

Experiment no.	No. of replicates	Solution	Without iodoacetate			With iodoacetate 2×10^{-5} M			Iodoacetate inhibition	Least significant difference	
			Length	Increase over control	Response to K	Length	Increase over control	Response to K			
			cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*0.05*	*0.01*
3	2	Water control	5.01	.....	....	3.41	....	....	1.60	0.37	0.51
		Sodium pyruvate 0.001 M	4.93	−0.08	....	3.78	0.37	....	1.15		
		" " 0.005 M	4.77	−0.24	....	3.86	0.45	....	0.91		
		Potassium pyruvate 0.001 M	5.73	0.72	0.80	4.46	1.05	0.68	1.27		
		" " 0.005 M	5.80	0.79	1.03	5.33	1.92	1.47	0.47		
		Sodium acetate 0.001 M	5.23	0.22	....	3.91	0.50	....	1.32		
		" " 0.005 M	4.91	−0.10	....	4.13	0.72	....	0.78		
		Potassium acetate 0.001 M	5.70	0.69	0.47	4.59	1.18	0.68	1.11		
		" " 0.005 M	5.82	0.81	0.91	5.02	1.61	0.89	0.80		
4	3	Water control	4.55	....	....	3.82	....	....	0.73	0.42	0.57
		Sodium malate 0.005 M	4.59	0.04	....	4.24	0.42	....	0.35		
		Potassium malate 0.005 M	5.19	0.61	0.60	4.95	1.13	0.71	0.21		
		Sodium succinate 0.005 M	4.68	0.13	....	4.04	0.22	....	0.64		
		Potassium succinate 0.005 M	5.16	0.61	0.48	4.82	1.00	0.78	0.34		
		Sodium nitrate 0.010 M	5.01	0.46	....	4.33	0.51	....	0.68		
		Potassium nitrate 0.010 M	5.56	1.01	0.55	4.95	1.13	0.62	0.61		

of various potassium salts show growth response in the coleoptile. The question might now be raised as to whether any of the salts employed alleviate iodoacetate inhibition. In the column headed iodoacetate inhibition in table I are shown differences in coleoptile lengths between plants grown in each salt alone and plants grown in the same salts in the presence of iodoacetate. The fiducial limits reported in table I are not applicable to comparisons between values for iodoacetate inhibition, since values for iodoacetate inhibition are differences between treatments and comparisons between these values are interactions. Although not tested statistically, alleviation of inhibition appears to have resulted in sodium and potassium salts of fumarate, sulphate, pyruvate, acetate, and malate, as well as potassium salts of malonate and succinate when supplied at the higher concentration (0.005 M or 0.01 M). Chlorides when supplied at 0.002 M appeared to accentuate iodoacetate inhibition, but at 0.01 M seemed to have no effect.

The influence of sodium and potassium salts upon growth of the first leaf

Lengths of the first leaf are shown in table II. Growth responses of the first leaf are similar to those of the coleoptile in these essential respects: In each experiment some reduction in growth resulted in 2×10^{-5} M iodoacetate. In the presence or absence of iodoacetate, each potassium salt used resulted in a highly significant response. Responses to inorganic potassium salts were of the same magnitude as responses to the organic potassium salts used. Sodium salts frequently resulted in significant growth responses, but these responses were smaller than those to potassium salts. Inorganic salts of sodium produced responses similar to those of organic salts of sodium.

The influence of sodium and potassium salts upon length of roots

The length of the longest root and the total length of roots were both determined in most cases. The responses to treatments shown by these two measurements were similar in those cases where both measurements were made. The length of the longest root is used as a criterion of root length, since complete data from all experiments were obtained for this measurement. The influence of various treatments upon the length of the longest root is shown in table II. Iodoacetate in a concentration of 2×10^{-5} M caused little or no inhibition in root growth. Sodium and potassium salts of fumarate, malonate, pyruvate and acetate at the lower concentration (0.001 M) resulted in increases in root length. These increases were not sustained at the concentration of 0.005 M except in the case of pyruvate. At the latter concentration, sulphates and malates resulted in significant inhibition. Chlorides had little or no effect on root length at the concentrations used. Iodoacetate at 2×10^{-5} M had no effect upon these responses. In only one case was there a significant difference between response to a potassium salt and response to the corresponding sodium salt (*i.e.*, 0.005 M

malonate in the absence of iodoacetate). This, however, appears to be an anomalous result, since no corresponding result occurred in the presence of iodoacetate. In all other cases root elongation appeared to be influenced only by the anion employed and by the concentration of this anion.

Discussion

The results of ALBAUM and COMMONER (**1**) and ALBAUM and EICHEL (**2**) were confirmed in this study to the extent that potassium salts of fumarate, pyruvate, malate, succinate and malonate resulted in increases in growth of the Avena coleoptile. This was the case both in the presence and absence of iodoacetate. However similar responses resulted from potassium salts of acetate, chloride, sulphate, and nitrate. Finally, the sodium salts of the corresponding acids gave no comparable responses. Clearly the principal growth responses in the coleoptile resulted from potassium. Among the sodium salts which resulted in significant responses, the inorganic salts equaled or excelled the organic sodium salts employed.

At a concentration of 0.005 M or 0.01 M the potassium salt of each of the organic acids used appeared to alleviate iodoacetate inhibition. However, this effect cannot be ascribed specifically to either the potassium ion or the organic anions used, since similar effects were obtained with sodium salts of some of the organic acids and with sodium and potassium sulphate.

Although an acid cycle of the type described by KREBS (**6**) is involved at least in part in the metabolism of the Avena coleoptile as shown by the work of BONNER (**3**), this could not be shown in terms of growth responses to salts of certain of these acids when supplied to the roots of the seedlings. The apparently anomalous result of ALBAUM and EICHEL (**2**) wherein malonate seemed capable of reversing iodoacetate inhibition may now be explained principally in terms of a response to the potassium used in neutralizing the acid, and in part by a salt effect. The salt effect is non-specific in nature, since it is produced by sulphates as well as by salts of organic acids. Similar results purporting to show reversal of iodoacetate inhibition by malonate and various acids of the Krebs cycle in isolated coleoptile sections have been reported by THIMANN and BONNER (**10**). A reinvestigation of these results will be reported in a separate paper.

Increases in root elongation resulted when salts of several organic acids were used in low concentrations. Sulphate and chloride salts did not produce increases. It is conceivable that these responses resulted from utilization of the acids in the metabolism of the roots. However, these results can hardly be attributed to influences exerted through a metabolic cycle involving succinic dehydrogenase, since malonate as well as other organic acids resulted in increased root elongation.

Summary

Young Avena seedlings were grown in solutions of sodium and potassium salts of several organic and inorganic acids in the presence and absence of

TABLE II

LENGTHS OF THE FIRST LEAF AND LONGEST ROOT OF SEEDLINGS OF *Avena sativa*, VAR. BLACK NORWAY GROWN IN VARIOUS SOLUTIONS.

Experiment no.	No. of replicates	Solution	Length of first leaf				Length of longest root			
			Without iodoacetate	With iodoacetate 2×10^{-3} M	Least significant difference		Without iodoacetate	With iodoacetate 2×10^{-3} M	Least significant difference	
			cm.	*cm.*	*0.05*	*0.01*	*cm.*	*cm.*	*0.05*	*0.01*
1	4	Water control	10.5	9.5	1.67	2.23	10.8	10.6	2.28	3.04
		Sodium fumarate 0.001 M	13.4	10.5			16.2	15.4		
		" " 0.005 M	11.9	9.7			8.7	8.7		
		Potassium fumarate 0.001 M	15.5	14.3			15.3	14.8		
		" " 0.005 M	16.2	16.5			8.8	9.3		
		Sodium chloride 0.002 M	14.2	11.2			12.2	12.2		
		" " 0.010 M	13.5	10.1			10.6	10.7		
		Potassium chloride 0.002 M	16.1	15.5			10.6	11.5		
		" " 0.010 M	17.3	16.3			10.7	12.6		
2	3	Water control	10.9	8.9	1.27	1.70	11.2	10.3	1.56	2.10
		Sodium malonate 0.001 M	13.2	10.6			15.9	14.7		
		" " 0.005 M	12.2	10.6			7.8	9.2		
		Potassium malonate 0.001 M	16.9	15.4			17.0	15.7		
		" " 0.005 M	16.9	16.3			10.5	8.9		
		Sodium sulphate 0.001 M	15.1	11.2			8.5	8.8		
		" " 0.005 M	13.1	9.9			5.3	6.0		
		Potassium sulphate 0.001 M	17.4	14.6			7.4	8.2		
		" " 0.005 M	16.6	15.4			5.9	5.7		

TABLE II *(continued)*

Experiment no.	No. of replicates	Solution	Length of first leaf				Length of longest root			
			Without iodoacetate	With iodoacetate 2×10^{-5} M	Least significant difference		Without iodoacetate	With iodoacetate 2×10^{-5} M	Least significant difference	
			cm.	*cm.*	*0.05*	*0.01*	*cm.*	*cm.*	*0.05*	*0.01*
3	2	Water control	12.4	8.3	2.05	2.81	10.1	9.2	1.88	2.58
		Sodium pyruvate 0.001 M	14.8	10.8			13.6	11.9		
		" " 0.005 M	11.8	11.6			13.2	13.1		
		Potassium pyruvate 0.001 M	17.9	14.8			12.8	13.0		
		" " 0.005 M	17.7	16.6			13.8	13.3		
		Sodium acetate 0.001 M	15.5	11.9			14.0	14.2		
		" " 0.005 M	13.4	10.2			9.7	9.2		
		Potassium acetate 0.001 M	17.9	13.5			13.5	12.5		
		" " 0.005 M	18.6	17.0			9.4	8.6		
4	3	Water control	10.4	8.8	1.69	2.29	10.8	10.9	2.59	3.50
		Sodium malate 0.005 M	12.5	10.7			4.5	4.6		
		Potassium malate 0.005 M	17.3	15.8			5.3	5.0		
		Sodium succinate 0.005 M	12.7	10.2			9.7	8.0		
		Potassium succinate 0.005 M	17.1	14.9			11.1	8.9		
		Sodium nitrate 0.010 M	14.6	11.4			9.5	11.1		
		Potassium nitrate 0.010 M	19.4	17.6			11.3	11.4		

iodoacetate. Growth of the coleoptile and first leaf was inhibited by iodoacetate but growth in length of the roots was not appreciably affected. In the coleoptile and first leaf large growth responses resulted from all the potassium salts in the presence and absence of iodoacetate. Some of the sodium salts resulted in smaller growth responses, but in this respect inorganic salts were as effective as any of the salts of organic acids used. Thus, it is shown that the growth stimulation of the coleoptile resulting from potassium salts of several organic acids in the presence of iodoacetate is primarily a response to potassium. In addition to the potassium response some salts have the effect of decreasing iodoacetate inhibition in the coleoptile. The latter effect is obtained with sulphates as well as salts of organic acids and is not, therefore, specific reversal of iodoacetate inhibition by organic anions.

Growth of the roots was influenced primarily by the particular anion and by the concentration of the anion supplied. Sodium and potassium salts of several organic acids supplied at low concentrations resulted in increased elongation of the roots. No comparable response was produced by chlorides or sulphates. Growth inhibition resulted when some of the salts were supplied in higher concentration.

Department of Plant Physiology
Hawaii Agricultural Experiment Station
Honolulu, Hawaii

LITERATURE CITED

1. Albaum, H. G. and Commoner, B. The relation between the four-carbon acids and the growth of oat seedlings. Biol. Bull. **80**: 314–323. 1941.
2. Albaum, H. G. and Eichel, B. The relationship between growth and metabolism in the oat seedling. Amer. Jour. Bot. **30**: 18–22. 1943.
3. Bonner, J. Biochemical mechanisms in the respiration of the Avena coleoptile. Arch. Biochem. **17**: 311–326. 1948.
4. Commoner, B. and Thimann, K. V. On the relation between growth and respiration in the Avena coleoptile. Jour. Gen. Physiol. **24**: 279–296. 1941.
5. Kaiser, S. and Albaum, H. G. Early root and shoot growth in two varieties of *Avena sativa* in relation to growth substances. Amer. Jour. Bot. **26**: 749–754. 1939.
6. Krebs, H. A. The intermediary stages in the biological oxidation of carbohydrate. Adv. in Enzymol. **3**: 191–252. 1943.
7. Robertson, W. B. The preparation of sodium pyruvate. Science **96**: 93–94. 1942.
8. Snedecor, G. W. Statistical Methods. Third Ed. Iowa State Col. Press, Ames. 1940.

9. SZENT-GYÖRGI, A. The significance of fumaric acid for the respiration of animal tissue. Introduction, scope and methods. Zeit. Physiol. Chem. **236**: 1–20. 1935.
10. THIMANN, K. V. and BONNER, W. D., JR. Experiments on the growth and inhibition of isolated plant parts. I. The action of iodoacetic and organic acids on the Avena coleoptile. Amer. Jour. Bot. **35**: 271–281. 1948.
11. THIMANN, K. V. and SCHNEIDER, C. L. The role of salts, hydrogen-ion concentrations and agar in the response of the Avena coleoptile to auxins. Amer. Jour. Bot. **25**: 270–280. 1938.
12. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Burgess Publishing Co., Minneapolis. 1948.

BRIEF PAPERS

ASCORBIC ACID AND ASCORBIC ACID OXIDIZING ENZYMES OF GREEN BEAN PLANTS DEFICIENT IN MANGANESE[1]

KATHARINE J. HIVON,[2] D. M. DOTY[3] AND F. W. QUACKENBUSH

Received November 10, 1950

Recently in this laboratory (**1**) soybeans grown on manganese-deficient field plots were analyzed for ascorbic acid and ascorbic acid-oxidizing enzymes. Contrary to some reports in the literature, no consistent relationship was found between manganese deficiency symptoms and ascorbic acid or the enzymes which oxidize it. The present study was performed with solution cultures to provide a more rigid deficiency of manganese.

Stringless green pod beans (Asgrow) were germinated in controlled temperature cabinets in paper rolls moistened with distilled water. Seedlings were transferred at 10 days to solution culture vessels. The continuous solution renewal method which automatically aerates the solution, as described by SHIVE and STAHL (**4**), was employed with some modification. Sixteen units of six culture vessels each were arranged on greenhouse benches by randomizing the order of three reservoir jars, one from each of the three experimental treatments. Each reservoir jar supplied two culture vessels.

Macronutrient elements were supplied in the amounts used by SOMERS *et al.* (**5**) in a study of the effects of iron–manganese ratios on soybean plants. In millimolar concentrations these were: KH_2PO_4—0.58, K_2SO_4—0.82, $Ca(NO_3)_2$—4.50, and $MgSO_4$—2.30. Iron as $FeSO_4$ (low Mn) and manganese as $MnSO_4$ were added in the ratios of 4.0 to 0.00, 4.0 to 0.01, and 4.0 to 2.00 (p.p.m.). Micronutrient elements other than manganese were supplied in the following amounts and forms: 1.0 p.p.m. zinc ($ZnSO_4$), 0.5 p.p.m. boron (H_3BO_3), and 0.1 p.p.m. copper ($CuSO_4$). Distilled water and analytical grade reagents were used throughout. The cultural solutions which were made each morning from concentrated reagent solutions were maintained at pH 4.0 to 4.2. Acidified ferrous sulphate solution was prepared fresh from the dry salt each morning immediately prior to addition to the cultural solutions.

The greenhouse temperature was maintained at 80° F in day and 70° F at night. Since the experiment was performed during the winter months, six hours of supplementary lighting from incandescent lamps was provided each clear day and thirteen hours on cloudy days. Parathion (*O, O*-diethyl

[1] Journal paper no. 489 of the Purdue University Agricultural Experiment Station.

[2] Present address: Dept. of Home Economics, Univ. of Illinois, Urbana.

[3] Present address: American Meat Institute, Chicago, Illinois.

O-p-nitrophenylthiophosphate) dust was applied to all solution-cultured plants at 36 and 50 days to control thrips and red spiders.

The determination of manganese in distilled water and in cultural solutions was made by a periodate method which combined and modified those of PEECH (2) and SANDELL (3). The Evelyn photoelectric colorimeter was used with filter no. 520. Plants were harvested at approximately the same morning hour in each sampling period. Stems were cut at the stem-root transition zone and samples were held in an ice-cooled, insulated chest until they could be placed in the electric refrigerator. Leaves and pods were removed within three hours after harvesting. Duplicate groups of plants

TABLE I

REDUCED ASCORBIC ACID CONTENT (mg./100 g.) OF GREEN BEAN PLANTS GROWN IN CULTURAL SOLUTION (DUPLICATE SAMPLES; DRY WEIGHT BASIS)

Plant part	Days from seed	Added manganese (p.p.m.)			L.S.D.	
		0.00	0.01	2.00	5%	1%
Leaves	27	447	480	506	*	*
	34	558	557	628	*	*
	41	514	572	487	*	*
	48	628	670	531	*	*
	55	466	497	358	*	*
	62	755	660	565	*	*
	69	....	632	576	*	*
Stems	27	401	348	371	*	*
	34	267	299	338	46	*
	41	232	250	229	*	*
	48	303	255	218	*	*
	55	174	178	164	*	*
	62	213	238	196	*	*
	69	....	243	192	*	*
Pods	48	282	...	423	76	*
	55	178	....	264	*	*

*The F test indicated nonsignificance; therefore the least significant difference was not calculated.

from each treatment were processed. In the first harvest 10 plants, in the second through fourth harvests eight plants, and in the remaining three harvests five plants comprised each duplicate sample. Weighed samples were autoclaved at 15 pounds pressure for eight minutes and dried for approximately 84 hours at 70° C in a vacuum oven. The only change from the method previously described (1) in the analysis for ascorbic acid was the addition of 1% ammonium sulphate to clarify leaf and pod extracts. The determination of enzymic ascorbic acid-oxidizing activity was made by the method previously described (1).

Although the manganese content of the distilled water was less than 0.01 p.p.m., all plants on the three levels of manganese developed similarly during the first four weeks of growth. At 27 days, the first sampling period,

a slight weight difference in the plants was positively related to manganese supplied. At 30 days the two groups on restricted manganese showed definite interveinal chlorosis of newer leaves. The deficiency symptoms of the plants became increasingly severe and pronounced chlorosis with loss of some leaves, growing points, blossoms, and pods occurred in both groups on restricted manganese. In contrast, the plants on the highest level of manganese grew vigorously and produced leaves of normal color.

Root growth was very poor in the plants receiving no added manganese. The upper portion of the main root and the rootlet tips were also markedly discolored. Roots of plants receiving 0.01 p.p.m. were longer but less

TABLE II

ASCORBIC ACID OXIDIZED BY LEAF ENZYMES (γ/100 mg. leaf) OF GREEN BEAN PLANTS GROWN IN CULTURAL SOLUTION (DUPLICATE SAMPLLS; DRY WEIGHT BASIS)

Plant part	Days from seed	Added manganese (p.p.m.)			L.S.D.	
		0.00	0.01	2.00	5%	1%
Leaves	27	3.4	6.9	6.2	*	*
	34	9.5	12.2	10.3	*	*
	41	12.6	9.9	17.1	2.8	*
	48	15.1	14.0	9.3	1.5	3.5
	55	12.7	12.4	10.0	*	*
	62	6.3	10.4	8.6	*	*
	69	....	12.0	8.6	*	*
Stems	27	18.2	17.1	16.6	*	*
	34	20.9	21.5	25.8	*	*
	41	23.8	25.0	26.6	*	*
	48	23.8	27.4	16.2	*	*
	55	28.8	25.5	16.5	*	*
	62	27.3	17.8	14.9	4.8	*
	69	....	14.6	25.4	*	*
Pods	48	21.1	....	23.0	*	*

*The F test indicated nonsignificance; therefore the least significant difference was not calculated.

branched than those receiving 2.00 p.p.m. There was no discoloration of roots of plants on the two higher levels of manganese.

No seed was produced by either of the two deficient groups of plants. However, these groups produced a similar number of small empty pods. Size of pods and seed formation was apparently normal for the plants grown in cultures to which 2.00 p.p.m. of manganese had been added.

In only two instances was a significant positive relationship observed between manganese content of the solutions and ascorbic acid concentration of the plant material when statistical analysis was applied to the data (table I). Noticeable variations in ascorbic acid content seemed to coincide with applications of parathion dust. However, in a separate experiment with parathion treatments, analyses for ascorbic acid of leaves showed no

statistically significant relationship between parathion treatment and ascorbic acid content.

The effect of sunlight was given attention since there is considerable evidence for a positive relationship between length of exposure and intensity of light and ascorbic acid of plants. Despite the supplemental artificial lighting supplied during the months of short photoperiod there was a definite positive relationship between sunlight and ascorbic acid content of the plants which appeared to explain the variations observed in the latter.

The results of enzyme activity tests, presented in table II, do not allow a conclusion to be drawn concerning a relationship between manganese content of the solutions and ascorbic acid-oxidizing activity of the plant. Enzyme activity of the plants did not seem to follow a consistent pattern of change over the portion of the growth period observed. The greater activity of stems over leaves cannot be explained on the basis of available substrate.

Since the manganese level did not consistently affect the amount of ascorbic acid or the ascorbic acid-oxidizing activity of the plant tissue studied, it does not seem likely that the essential nature of manganese for plant growth is related to reactions involving formation or oxidation of ascorbic acid.

Department of Agricultural Chemistry
Purdue University
Lafayette, Indiana

LITERATURE CITED

1. Hivon, Katharine J., Doty, D. M., and Quackenbush, F. W. Ascorbic acid and ascorbic acid-oxidizing enzymes of manganese-deficient soybean plants grown in the field. Soil Sci. **71**: 353–359. 1951.
2. Peech, M. Determination of exchangeable bases in soils. Ind. and Eng. Chem., Anal. Ed. **13**: 436–441. 1941.
3. Sandell, E. B. Chemical Analysis. Vol. 3. Colorimetric Determination of Traces of Metals. Interscience Publishers, Inc., New York. 1944.
4. Shive, J. W. and Stahl, A. L. Constant rates of continuous solution renewal for plants in water culture. Bot. Gaz. **84**: 317–323. 1927.
5. Somers, I. I., Gilbert, S. G., and Shive, J. W. The iron–manganese ratio in relation to the respiratory carbon dioxide and deficiency-toxicity symptoms in soybeans. Plant Physiol. **17**: 317–320. 1942.

SOME RESPONSES OF YOUNG CORN PLANTS TO MALEIC HYDRAZIDE[1]

LOYD A. TATUM AND JOHN H. CURME

(WITH ONE FIGURE)

Received March 26, 1951

Maleic hydrazide was reported by SCHOENE and HOFFMAN (**6**) to have an unusual effect on the growth of plants. The appearance of several recent publications (**1, 2, 4, 5, 7, 8, 9**) describing the effects of the chemical on plants indicates considerable interest in its properties and possible applications as a plant-growth regulant. The property of maleic hydrazide for retarding growth temporarily suggested the possibility of using it to delay one of the parents in hybrid corn seed production fields to bring parents of different maturity into flower at the same time, thus avoiding the necessity of split planting dates. The following is a report of some results obtained when the chemical was sprayed on young corn plants.

The material treated was a planting of several inbred and single cross strains made on June 21, 1949. On July 16 when the plants were 25 days old and the tallest leaves could be extended to a height of 50 to 75 centimeters, a 30% formulation of the diethanolamine salt of maleic hydrazide supplied by Naugatuck Chemical Division, U. S. Rubber Company, Naugatuck, Connecticut, was sprayed into the leaf whorl from a knapsack sprayer. Concentrations used were 1000, 2000, 4000, and 8000 parts per million of maleic hydrazide with "Dreft" as a wetting agent. Five plants of each strain were treated with each of the concentrations. Each plant received approximately equal doses but no attempt was made to standardize the application on the basis of rate per acre. Groups of five untreated plants alternated in the row with the various treatments. Individual plant heights were recorded 3, 11, 17, and 25 days after treatment. Typical results are shown graphically in figure 1.

A pronounced differential response of the various strains of corn to the chemical and to different concentrations of it was apparent. With the exception of K155 × K201 during the 11 to 17 day period, the 1000 p.p.m. concentration inhibited growth in all strains throughout the period during which measurements were taken. SA24, an inbred line of popcorn, seemed to be the most sensitive strain tested, particularly during the second and third periods of growth. At 2000 p.p.m. several strains responded very much as

[1] Contribution no. 430, Department of Agronomy, Kansas Agricultural Experiment Station. Cooperative investigations of Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Kansas Agricultural Experiment Station.

they had at 1000 p.p.m., but the inhibition on others was more than twice as great as at the lower concentration. At 4000 p.p.m. the degree of inhibition was much greater and differences between strains less distinct than at 2000 p.p.m., but those strains giving some indication of tolerance at 4000 p.p.m. were among the tolerant strains also at 2900 p.p.m. The 8000 p.p.m. treatment almost completely prevented growth during the first period after application, and most plants were dead before the last measurement was made.

Other than retarded growth, the first response noted was the development of red pigment in the leaves. This was most pronounced in the plots that had received the higher concentrations. The appearance was similar

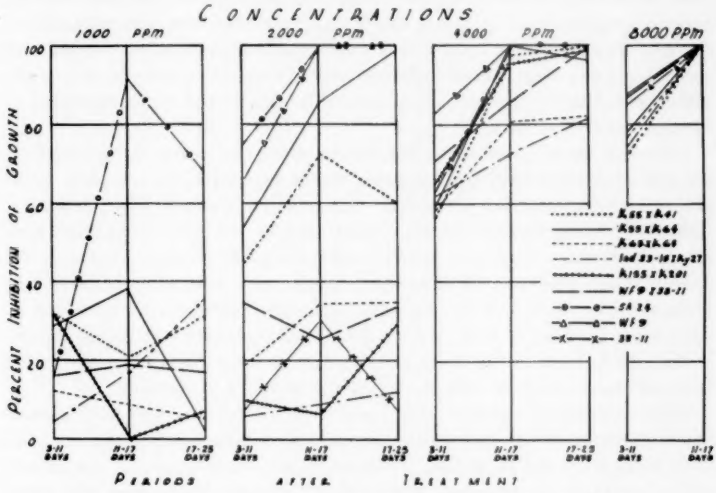


FIG. 1. Inhibition of growth in corn sprayed with maleic hydrazide.

to that of barren plants, which have high sugar accumulation in vegetative tissues.

A prolonged dry period which occurred following treatment of the plots probably had considerable effect on the results. It was noted that the treated plants wilted sooner and fired more severely than the untreated plants. The increase in susceptibility to drought corresponded roughly to the extent to which growth had been inhibited. It was most severe at the higher concentrations and on the strains showing the most response to the chemical.

Flowering was affected so adversely by the dry weather that it was not felt that dependable comparisons of dates could be made. It was retarded on the treated plots but probably more because of their greater susceptibility to drought than because of growth inhibition due to maleic hydrazide.

As the tassels began to emerge it was apparent that those on some strains had been affected in a striking manner while those on other strains were almost normal. The abnormality ranged from one or two aborted branches to the more extreme condition in which only a stub was produced. Pollen was produced by the less seriously affected tassels. In addition to the effects on the tassels, the upper leaves in some cases were narrow and abnormal in appearance.

The treatment, on the basis of this preliminary test, does not seem to offer much promise as a practical means of controlling flowering dates in corn but is extremely interesting because of its effect on the development of the plants. The development of pigment in the leaves may have resulted from the accumulation of sugars which normally would have been utilized in growth processes. It seems likely that the increased susceptibility to drought was due to the lack of new root growth which limited the soil volume and depth from which moisture could be drawn. Periods of drought are expected in most seasons in Kansas, and use of the chemical would be hazardous for that reason.

The differential response of strains of corn to the chemical is interesting. At first it seemed that the chemical might be extremely selective in its action, and that is still a possibility. However, it was noted that generally, though not without exception, the strains showing the greatest response were the earlier ones. That may indicate that the stage of development at the time of treatment was an important factor. As shown by MARTIN and HERSHEY (**3**), all the parts of a corn plant are differentiated by about 30 days after planting. With late planting under Kansas conditions, it may be that 25-day old plants of the early strains in the present experiment were more advanced than the plants studied by Martin and Hershey.

The differential response may have occurred because the early strains had differentiated all their structures and were approaching the stage of rapid elongation and expansion, whereas the later ones were still in a formative stage when the chemical was applied. If such is the case, the tassel abnormalities might be avoided by treating seedlings at an earlier stage or by applying the chemical to the seed before planting. Weed control would be a difficult problem, though, if the treatment were made before the corn plants were large enough to be cultivated. Another possibility would be to treat the plants after the period of tassel differentiation is completed, but the results of NAYLOR (**5**) suggest that this may result in male sterility.

DEPARTMENT OF AGRONOMY
KANSAS AGRICULTURAL EXPERIMENT STATION
MANHATTAN, KANSAS
AND
DIVISION OF CEREAL CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING
AGRICULTURAL RESEARCH ADMINISTRATION
UNITED STATES DEPARTMENT OF AGRICULTURE

LITERATURE CITED

1. Carrier, H. B. and Crafts, A. S. Maleic hydrazide, a selective herbicide. Science **111**: 152–153. 1950.
2. Fillmore, R. H. The control of plant development with maleic hydrazide. Arnoldia **10**: 33–38. 1950.
3. Martin, J. N. and Hershey, A. L. The ontogeny of the maize plant—the early differentiation of stem and root structures and their morphological relations. Iowa State Col. Jour. Sci. **9**: 489–503. 1935.
4. Moore, R. H. Several effects of maleic hydrazide on plants. Science **112**: 52–53. 1950.
5. Naylor, A. W. Observations on the effects of maleic hydrazide on the flowering of tobacco, maize and cocklebur. Proc. Nat. Acad. Sci. **36**: 230–232. 1950.
6. Schoene, D. L. and Hoffman, O. L. Maleic hydrazide, a unique growth regulant. Science **109**: 588–589. 1949.
7. White, D. G. Blossoming of fruits delayed by maleic hydrazide. Science **111**: 303. 1950.
8. Wittwer, S. H. and Sharma, R. C. The control of storage sprouting in onions by preharvest foliage sprays of maleic hydrazide. Science **112**: 597–598. 1950.
9. Zukel, John W. Use of maleic hydrazide as a plant growth inhibitor. Agr. Chem. **5**: 35–36. 1950.

A TECHNIQUE FOR GROWING PLANTS UNDER STERILE CONDITIONS

FRANK GERMAN AND VAUGHAN T. BOWEN

(WITH ONE FIGURE)

Received January 22, 1951

In a study of albinism in corn, SPOEHR (**3**) succeeded in raising albino seedlings to maturity, by feeding them sucrose solution through the cut ends of leaves. In our laboratory a similar attempt on an albino strain obtained from EVERETT (**2**), proved unsuccessful, the seedlings showing very slow growth, and dying at a height of about six inches. Since the appearance of the plants indicated an inadequate carbon supply, and it was desired to eliminate the laborious task of changing the nutrient solution daily, a modification of the sterile flask-culture technique (**4**) was attempted. For such studies the strain used is especially useful since the seed pigmentation indicates albinos, permitting their selection before germination.

The apparatus, assembled, is illustrated in figure 1. The air pump and bubble-lift pump served to circulate the culture solution and to provide atmosphere circulation as well. Adequate air sterilization was provided by the Koby air filter, a patented device obtained from the Koby Corporation, Boston, Massachusetts. For a seed bed a short, wide length of Pyrex tubing was filled with washed gravel, and the ends enclosed in cotton gauze. The corn root soon penetrated this to the level of the nutrient solution, making unnecessary the cascading of nutrient over the gravel, which was the original purpose of the bubble-lift pump. The seeds were sterilized by immersion for 15 minutes in a 1/1000 solution of $AgNO_3$, and then rinsed three times in sterile water.

Autoclaving was carried out in two steps: The assembled apparatus, including the air filter, and containing 150 ml. of Ohio WP nutrient solution, was autoclaved 20 minutes at 20 lbs. pressure; and 150 ml. of 0.6 M sucrose solution (made with water previously autoclaved at 20 lbs. pressure, 20 minutes) was autoclaved 20 minutes at 10 lbs. pressure. After cooling and under carefully maintained sterile conditions, the sucrose solution was poured into the flask, the seed was placed on top of the gravel, and the stopper was put in place. An air flow of five to ten bubbles a minute was maintained from the electric aquarium pump, by means of a bleeder in the line (not shown).

For long term studies of plants which do not outgrow the sterile volume, provision must be made initially for change of air filters, as these have a specified life of only 30 days. As many filters as indicated have been placed in parallel, autoclaved at once, and those not in use cut off by pinch clamps.

Again for long term studies, calculation must be made of the amount of nutrients required. Should this amount exceed the capacity, at practical concentrations, of the flask, provision can be made for addition of nutrients by placing solids or strong solutions in tubes within the flask and using magnetically-controlled bead valves to permit later mixing. In our experiments, this has not yet been necessary.

Cultured in full sunlight in the greenhouse in August, the albino corn strain under investigation has been grown to the maximum size permitted

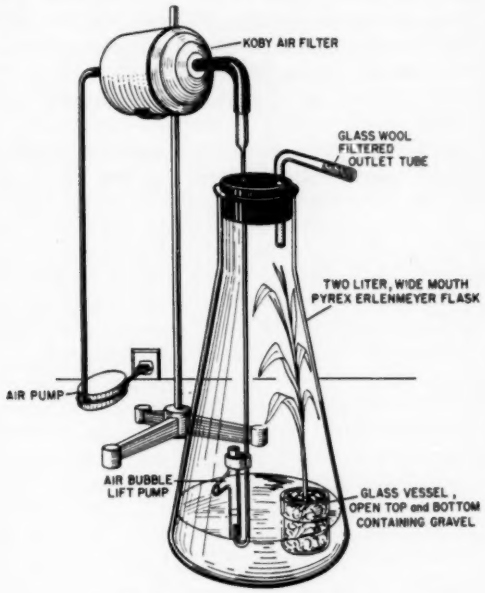

KOBY AIR FILTER
GLASS WOOL FILTERED OUTLET TUBE
TWO LITER, WIDE MOUTH PYREX ERLENMEYER FLASK
AIR PUMP
AIR BUBBLE LIFT PUMP
GLASS VESSEL, OPEN TOP and BOTTOM CONTAINING GRAVEL

FIG. 1. Diagram of apparatus.

by a two liter flask. During this period of growth (height of 30 cm.), no significant difference in rate of increase in length was observed between the cultured albinos and normal seedlings growing on soil in the greenhouse. The dry weight of the albinos was, however, only about half that of the soil controls. Experiments are now in progress to ascertain the cause of this weight discrepancy, and, using a much larger container, to measure the growth rate over a longer period.

BLANCHARD and DILLER (1) describe a method of raising water culture plants with only the roots sterile. It appears that both methods will prove useful, depending on the purposes of given experiments.

It is a pleasure to acknowledge the advice of Mr. Paul Steinglass on the practice of sterile technique, and the service of Mr. Henry Wright in preparing the figure.

BIOLOGY DEPARTMENT
BROOKHAVEN NATIONAL LABORATORY
UPTON, LONG ISLAND, NEW YORK

LITERATURE CITED

1. BLANCHARD, F. A. and DILLER, V. M. Technique for growing plants with roots in a sterile medium. Plant Physiol. **25**: 767–769. 1950.
2. EVERETT, H. Personal communication, Connecticut State Agr. Exper. Sta., New Haven, Connecticut. 1949.
3. SPOEHR, H. A. The culture of albino maize. Plant Physiol. **17**: 397–410. 1942.
4. STEINBERG, R. A. Growth responses to organic compounds by tobacco seedlings in aseptic cultures. Jour. Agr. Res. **75**: 81–92. 1947.

A TECHNIQUE FOR THE CULTIVATION OF HIGHER PLANTS UNDER STERILE CONDITIONS

HENRY R. KATHREIN

(WITH TWO FIGURES)

Received February 12, 1951

To study the effects of microörganisms of the soil on plant growth, it is essential that methods be developed by which plants can be grown under sterile conditions. FRED (**2**) has reported the use of a modification of KELLERMAN'S (**5**) system by which peas were grown in soil under sterile conditions. This method entailed the use of a glass cylinder which fitted onto a specially constructed metal pot in which the plant was to be grown. A glass beaker was inverted over the cylinder to prevent contamination. In a method developed by GERRETSEN (**3, 4**) oats, mustard, and sunflowers were grown in quartz sand under sterile conditions. Sterility of the pots was maintained by covering the sand with a layer of sterile cork filings mixed with katadyne silver. In both methods described, the nutrient salts were added directly to the pot before sterilization.

REUSZER (**6**) has described a method of cultivating squash and sunflowers under sterile conditions. In this method, the seed was placed in a vermiculite plant tube and the root allowed to grow down into a chamber containing a nutrient solution. A similar technique for growing plants with their roots in a sterile medium has been presented by BLANCHARD and DILLER (**1**). This method involved the placing of the seed in an aluminum column filled with vermiculite. Upon germination, the root penetrated a gauze layer into a sterile nutrient solution.

Studies on the nutrition of plants are being conducted in this laboratory by the use of sand culture. The plants are watered with nutrient solutions of known composition and flushed periodically with distilled water to prevent salt accumulation. None of the above methods were completely applicable to this method of raising plants; therefore, the technique described in this paper was developed. This technique involves the culturing of higher plants in pure quartz sand in such a manner that the entire plant is held under completely sterile conditions until maturity is reached.

Seeds were sterilized in a small filter flask using a 0.1% mercuric chloride solution. The period of sterilization varied with the type of seed to be sterilized; in the case of oats, immersion for a four minute period was sufficient to destroy the microörganisms present. During the sterilization period, a vacuum was alternately applied and released; and the flask was shaken to remove air bubbles from the surface of the seed. The seeds were then washed four or five times with sterile distilled water to remove any trace of the mercuric chloride. Following this treatment, the seeds were transferred to a bacteriological agar and allowed to germinate. Contamination was checked

by the sterility of the agar medium on which the seeds were placed. All seeds which showed contamination were discarded. When the roots of the plants reached an elongation of approximately five mm., the seeds were transferred aseptically with forceps to the sterile pots of sand.

Details of the apparatus described below are shown in figure 1. A standard four inch glazed soil jar with a hole in the side near the base was used

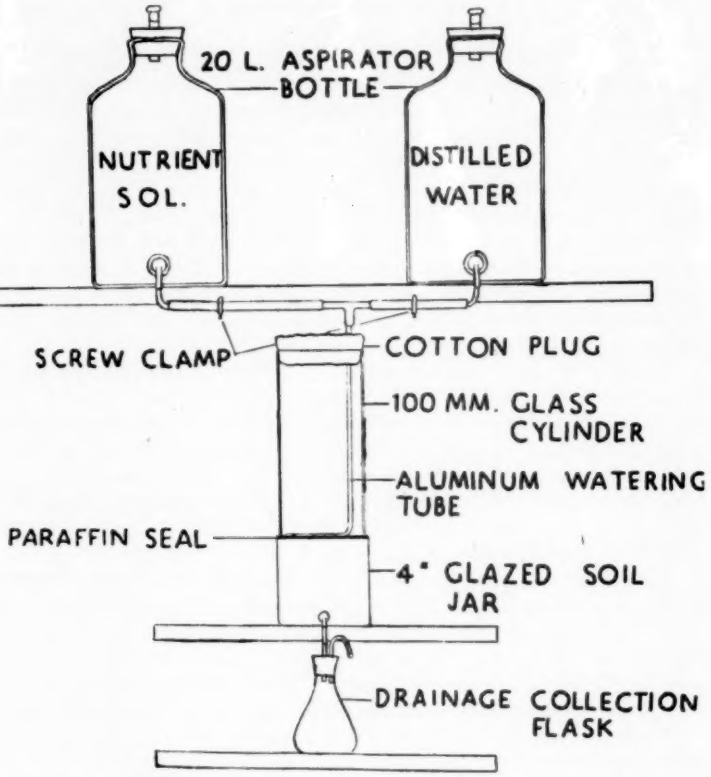


FIG. 1. Apparatus used for the culturing of higher plants under sterile conditions.

as a culture chamber. A rubber stopper with a glass tube passing through it was inserted into the hole at the base of the pot. To permit the drainage of excess nutrient solution, this glass tube was connected to a 1000 ml. Erlenmeyer flask by means of rubber tubing. A glass cylinder 100 mm. in diameter and 400 to 600 mm. in length and stoppered at the top with a large cotton plug was fitted onto the top of the soil jar and held in place by a paraffin seal. A piece of ¼ inch aluminum tubing wound tightly between the layers

of the cotton plug was passed down the cylinder into the soil jar. The aluminum tubing was made with a three inch loop on the soil jar end with holes drilled at one inch intervals on the loop. This tubing allowed the plant to be watered directly at the surface of the sand without washing the sand from around the roots of the plants. The top of the aluminum tubing was connected to a bottle of sterile nutrient solution and a bottle of sterile distilled water with rubber tubing. This allowed the soil jars to be watered with nutrient solution and flushed with distilled water. The bottles used for nutrient solution and distilled water were 20-liter aspirator bottles. Any number of soil jars may be connected to one nutrient solution bottle and one distilled water bottle by means of glass T connections. Circulation of air inside the glass cylinders may be accomplished by adding another T connection and forcing air through a cotton stoppered tube into the aluminum watering tube.

The soil jars containing the quartz sand were covered with a layer of gauze, cotton, and kraft paper tied in place. The rubber connections of the drainage tubes at the bottom of the jars were also wrapped with gauze, cotton and paper covers. The jars were then sterilized in an autoclave for three hours at 121° C, 15 pounds steam pressure to insure complete sterilization.

The bottoms of the glass cylinders were covered with gauze, cotton, and paper wrappers tied in place. All other connections of the apparatus were covered with gauze, cotton, and paper wrappers to maintain sterility after autoclaving. The glass cylinders, drainage bottles, and all T connections were autoclaved 15 minutes at 15 pounds of pressure. Because of their size, the 20-liter aspirator bottles which were used for nutrient solutions and distilled water were sterilized by dry heat (180° C, one and a half hours). The top and bottom openings were plugged with cotton. Following the sterilizing process, these plugs were replaced with rubber stoppers which had glass tubing connections. The stoppers and connections were sterilized in an autoclave. The nutrient solutions and distilled water were sterilized by passing them through a size A, normal porosity Berkefeld filter.

After the seeds had been sterilized and the proper stage of germination reached, they were transferred aseptically to the soil jars. The paper, cotton, and gauze wrapper was loosened from the top of the soil jar; and the seeds were positioned in the sand by means of sterile forceps. The wrappings were then removed from the glass cylinder and jar, and the cylinder was placed on top of the jar. The cylinder was sealed to the jar immediately with sterile paraffin. The paraffin was applied with a half inch brush that had been sterilized with the paraffin in a small Erlenmeyer flask. The aluminum tubing emerging from the cotton plug at the top of the cylinder was then connected to the nutrient solution and distilled water bottles by removing the wrappings and making the connection. The connection from the bottom of the soil jar to the collecting Erlenmeyer flasks was made in the same manner. The apparatus was then completely assembled, and the seeds were watered with distilled water. The nutrient solution and distilled water

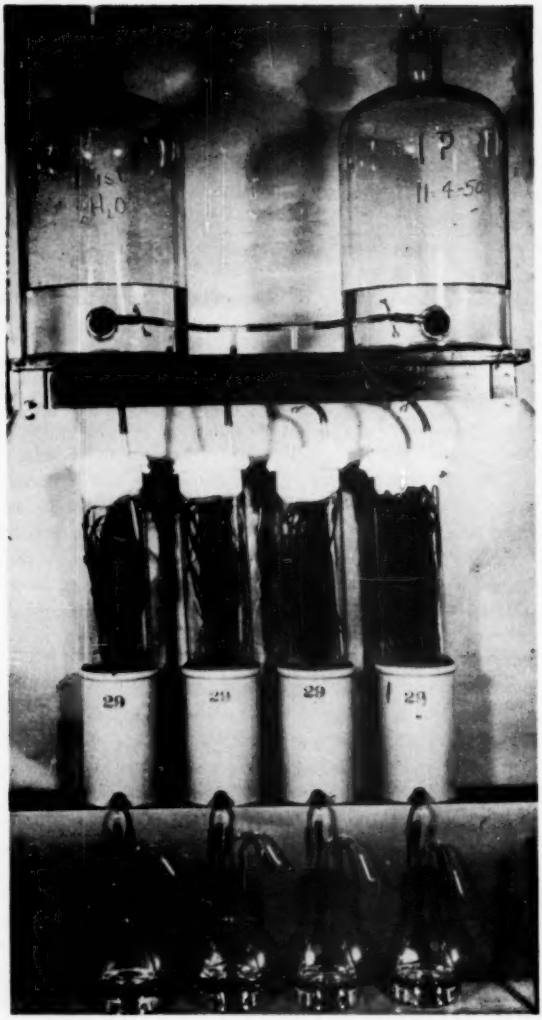


FIG. 2. Oat plants which have been grown under completely sterile conditions.

were controlled by means of screw clamps which enabled the watering with nutrient solution or the flushing of the jars with distilled water. Sterility of the soil jars was checked periodically by removing the drainage bottles and plating the nutrient solution with various bacteriological media.

Complete sterility was maintained on oat plants over a period of eight weeks after which time they were harvested for analysis. Average height of these plants was 500 mm. Figure 2 shows a set of oat plants raised under sterile conditions by the above method.

THE SAMUEL ROBERTS NOBLE FOUNDATION
ARDMORE, OKLAHOMA

LITERATURE CITED

1. BLANCHARD, FRED A. and DILLER, VIOLET M. Technique for growing plants with roots in a sterile medium. Plant Physiol. **25**: 767–769. 1950.
2. FRED, E. B. The growth of higher plants in soil free of microörganisms. Jour. Gen. Physiol. **1**: 623–629. 1919.
3. GERRETSEN, F. C. Das Katadyn Verfahren zur sterilen Kultur hoherer Pflanzen. Planta **23**: 593–605. 1935.
4. GERRETSEN, F. C. The influence of microörganisms on the phosphate intake of the plant. Plant and Soil **1**: 51–81. 1948.
5. KELLERMAN, K. F. U. S. Dept. Agr., Bur. Plant Ind., Bull. 120. 1914.
6. REUSZER, HERBERT W. A method for determining the carbon dioxide production of sterile and non-sterile root systems. Soil Sci. Soc. Amer. Proc. **14**: 175–179. 1949.

THE EFFECT OF MALEIC HYDRAZIDE ON TOMATO PLANTS IN RELATION TO THEIR AGE AT THE TIME OF TREATMENT

VICTOR A. GREULACH

(WITH TWO FIGURES)

Received March 26, 1951

Although it is known that most growth inhibitors affect young plants more severely than older ones, and random observations have been made of this effect of maleic hydrazide (MH), there has been no systematic investigation of the effect of MH on plants of different ages. Since information of this type has both a practical and theoretical value, it appeared to be worth while to investigate the phenomenon more thoroughly. CURRIER and CRAFTS (**1**) reported that the age of cotton and grass plants at the time of treatment affected their responses to MH, and NAYLOR and DAVIS (**5**) noted that "there is some loss in sensitivity with age, though not very great." The writer (**3**) has suggested that the more severe symptoms of MH treatment in tomatoes reported by him (**2**) compared with those reported by SCHOENE and HOFFMANN (**6**) and NAYLOR and DAVIS (**5**), including inhibition of leaf growth and stem diameter growth, were probably due to a difference in the age of the plants at the time of treatment.

On September 27, 1950, Earliana tomato plants three weeks old were selected for uniformity and divided into six lots of 10 plants each, one plant per pot. One lot was retained as a control group; one was sprayed with 2000 p.p.m. of the diethanolamine salt of maleic hydrazide, and the remaining lots were similarly sprayed at intervals of one week. The plants were maintained under good greenhouse cultural conditions. Weekly measurements of height and stem diameter were made, the latter with vernier calipers; and data on the general condition and development of the plants were taken. The experiment was discontinued on December 6, the soil was carefully washed from the roots, and fresh weights were determined. The plants were then dried at 70° C; and after dry weight determinations, the tissue was ground in a Wiley mill and retained for chemical analyses, which will be reported elsewhere.

Complete growth inhibition of the plants treated when three or four weeks old occurred within two weeks after treatment, within three weeks for plants five or six weeks old at treatment, and within four weeks for plants seven weeks old. Figure 1 shows that the amount of growth between treatment and complete cessation of growth increased with age at treatment, and for the ages used this relationship was linear. Growth curves for the plants are shown in figure 2 B. Figure 2 A illustrates the fact that MH inhibited the increase in stem diameter of plants treated when three, four, or five

weeks old in proportion to their age at treatment but did not inhibit the increase in stem diameter of plants treated when six or seven weeks old. No stem swelling as a result of MH treatment such as that found by NAYLOR and DAVIS (5) was observed; although stem swelling has been observed by the writer in other experiments with tomato, bean, and sunflower plants.

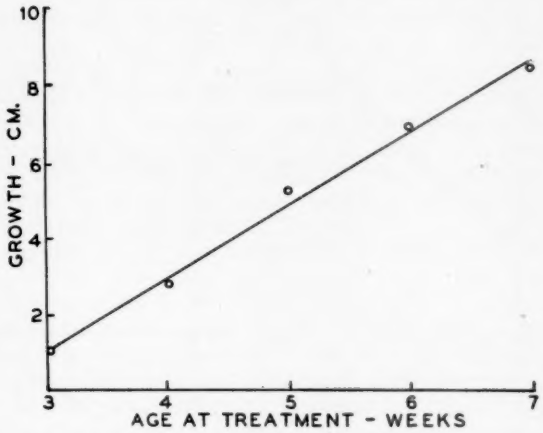


FIG. 1. Amount of growth of tomato plants from the date of treatment with maleic hydrazide until complete cessation of growth in height.

Table I illustrates the fact that all treated plants had higher shoot/root ratios than the controls, the extremely high S/R ratios of the plants treated when three weeks old being particularly noteworthy. While these ratios may indicate more effect of MH on root growth than on stem growth, it appears more likely that the marked inhibition of root growth was due to interference with translocation of food to the roots. This possibility is indicated by the high percentage water content of the roots of plants when three

TABLE I

EFFECT OF MALEIC HYDRAZIDE ON THE SHOOT/ROOT RATIOS AND WATER CONTENT OF TOMATO PLANTS TREATED AT DIFFERENT AGES

Age in weeks at time of treatment	Fresh weight, grams			Dry weight, grams			Per cent. water content	
	Shoots	Roots	Ratio	Shoots	Roots	Ratio	Shoots	Roots
3	1.06	0.03	35.33	0.13	0.003	43.33	87.7	90.0
4	3.82	0.61	6.26	0.43	0.07	6.14	88.7	88.5
5	12.03	1.65	7.30	1.55	0.26	5.95	87.1	84.2
6	16.40	2.80	5.86	2.01	0.44	4.57	87.7	84.3
7	29.93	4.41	6.54	3.19	0.63	5.06	89.1	85.7
Controls	39.09	10.57	3.75	3.39	1.60	2.12	91.3	84.8

or four weeks old, by the relatively low water content of the shoots of treated plants, by the report of McIlrath (4) that MH collapses the phloem elements in cotton, by the observations (unpublished) of the writer

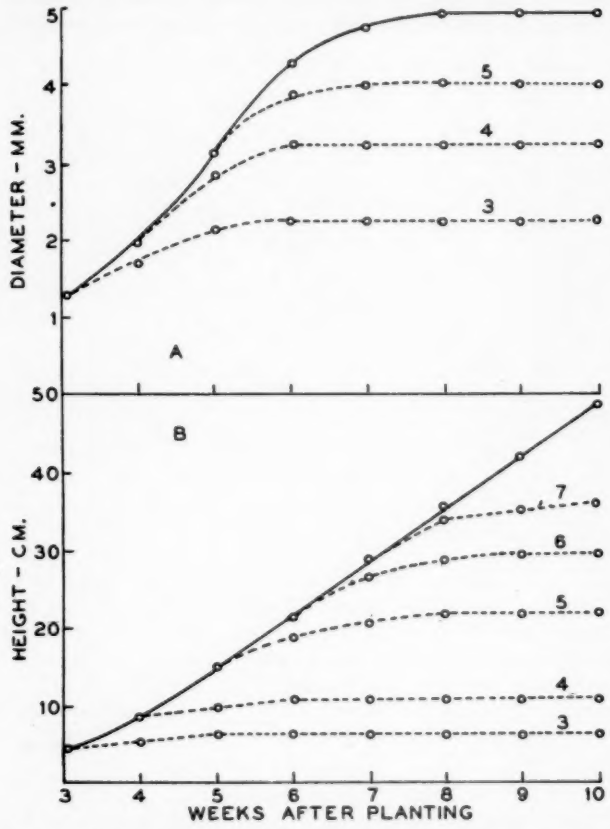


Fig. 2. A. Effect of maleic hydrazide on growth in diameter of tomato stems. Solid line represents growth of controls and plants treated at six and seven weeks of age, dotted lines the growth of plants treated when three, four, and five weeks old. B. Effect of MH on growth in height of tomato plants. Solid line represents growth of controls, and the figures on the dotted lines give age in weeks at time of treatment.

that translocation of starch from the leaves of treated tomato, bean, and sunflower plants is extremely slow, and by the reports of food accumulation in the leaves of treated plants.

The two leaves of the plants three weeks old never grew more than about

15% of the length of the comparable leaves of the controls, and the leaves of the plants four weeks old did not grow more than 30%. No additional leaves developed on plants in either of these groups. The MH did not inhibit growth of the older leaves in any other treatment, nor the growth of any leaves of the plant six or seven weeks old; but it did inhibit the young leaves of the plants five weeks old to about 75% of the comparable control leaves. The leaves on the plants three and four weeks old had only a few broad obtuse entire lobes while the older leaves of the other treated plants were of the usual shape. However, the leaves of these plants which developed subsequent to treatment had unusually narrow, highly divided acute lobes. The leaf blades on all treated plants rolled upward and inward at their margins, the degree decreasing with age at treatment. The leaves of the plants three weeks old first bent up and then slowly down, the axillary angle being about 20° two weeks after treatment, 10° three weeks after treatment, and 90° five weeks after treatment. The leaves of the plants four weeks old bent similarly, but no plant in any other treatment exhibited these responses.

Chlorosis was severe in the plants three weeks old, moderate in the plants four weeks old, and slight in the other treatment groups. It was preceded by an unusually dark green color. When a treated plant developed a branch of any size, its leaves were not chlorotic despite the condition of the leaves of the main stem. Anthocyanin appeared in the leaves of the plants three weeks old within a week after treatment; and in two weeks, the entire under surface of all leaves was a deep solid red color as were the veins on the upper surface and stems. In the plants four weeks old, anthocyanin distribution was similar but less intense and did not appear until two weeks after treatment. In the plants five weeks old, anthocyanin appeared on the veins only after three weeks; and in the groups six and seven weeks old, slight coloration of the veins appeared after four weeks.

By the end of the experiment, the controls were blooming freely; and the plants seven weeks old averaged 0.4 flowers plus flower buds per plant, but there were no flowers or macroscopic flower buds on plants in any other treatment group. Branch development was completely inhibited in the plants three weeks old; but the mean number of branches per plant in the groups four to seven weeks old was 2.1, 2.9, 2.8, and 2.5 in order. The control plants had just begun to develop a few young branches at the end of the experiment, evidently as a sequel of flowering.

On the basis of these data and observations, it seems safe to conclude that the difference in ages of the tomato plants at the time of treatment with MH has a very marked effect on the responses of the plants to the treatment and can readily account for discrepancies in previous reports on the effects of MH on tomatoes. These data also emphasize the importance of the age factor in conducting and interpreting experiments with MH and in the practical use of MH.

This report is based on a portion of a research program aided by a grant from the Carnegie Foundation for the Advancement of Teaching, making possible the employment of Mrs. Miriam Barteaux as a research assistant.

DEPARTMENT OF BOTANY
UNIVERSITY OF NORTH CAROLINA
CHAPEL HILL, NORTH CAROLINA

LITERATURE CITED

1. CURRIER, H. B. and CRAFTS, A. S. Maleic hydrazide, a selective herbicide. Science **111**: 152–153. 1950.
2. GREULACH, VICTOR A. Growth inhibition and injury of plants by maleic hydrazide. Texas Jour. Sci. **2**: 219–221. 1950.
3. GREULACH, VICTOR A. The effects of various concentrations of maleic hydrazide on tomato and etiolated bean plants. Texas Jour. Sci. **3**: 322–325. 1951.
4. MCILRATH, WAYNE J. Response of the cotton plant to maleic hydrazide. Amer. Jour. Bot. **37**: 816–819. 1950.
5. NAYLOR, A. W. and DAVIS, A. A. Maleic hydrazide as a plant growth inhibitor. Bot. Gaz. **112**: 112–126. 1950.
6. SCHOENE, D. L. and HOFFMANN, O. L. Maleic hydrazide, a unique growth regulant. Science **109**: 588–590. 1949.

NOTES

Southern Section.—The officers elected by the Southern section of the A. S. P. P. for 1951–1952 are: Chairman, G. M. Shear, Virginia Agricultural Experiment Station; Vice-Chairman, Howard B. Sprague, Texas Research Foundation; Secretary, D. E. Davis, Alabama Polytechnic Institute; Directors, Henry C. Harris, Florida Agricultural Experiment Station, John B. Whitney, Clemson Agricultural College, L. L. Danielson, Virginia Truck Experiment Station, E. M. Emmert, University of Kentucky.

Organization of a Washington, D. C., Section.—Plant physiologists of the Washington district area, including the district of Columbia and nearby Maryland and Virginia, have petitioned the A. S. P. P. for approval of a Washington area section. At the organizational meeting held January 22, 1951, it was decided to have a minimum of four regular meetings per year and to function on an academic year of September to June. From January to July, C. E. Minarik served as Chairman and R. B. Withrow as Secretary-Treasurer. Meetings were held at the Smithsonian Institution, in the Botany Department at the University of Maryland, and at the McCollum Pratt Institute of Johns Hopkins University. Recently elected officers for 1951–1952 are: Harry A. Borthwick, Chairman; Marion W. Parker, Vice-Chairman; and Robert B. Withrow, Secretary-Treasurer.

Leaf Analysis.—H. Lundegardh. Translated from the German by R. L. Mitchell. Hilger and Watts Ltd., 98 St. Pancras Way, Camden Road, London, N. W. 1. Obtainable from Jarrell-Ash Co., 165 Newbury Street, Boston 16, Massachusetts. 176 pages, 1951. $4.75.

The original monograph was published in 1945 but the translation has been made from a revised manuscript of December 1946. It describes the analysis of leaves as a method of determining the availability of nutrients in the soil and also an automatic flame-spectrographic unit used for analyses of large numbers of solutions. The first chapter of the monograph considers the scientific principles of leaf analysis and describes in detail many of the experiments performed in the author's laboratory on ion uptake and the distribution of mineral elements in the plant. The second chapter describes the sampling procedure, methods of analysis, and the effects on yield following application of potassium, phosphorus, and nitrogen to the soil in amounts determined by the analysis of the leaves. A third chapter discusses the determination of the fertilizer requirement of the soil by means of leaf analysis. A bibliography, an author index, and a subject index complete the volume.

Nitrogen in the Life of Plants.—Dimitrii N. Prianishnikov. Translated from the Russian by S. A. Wilde. A mimeoprint. Kramer Business Service, Inc., 16 N. Carroll Street, Madison, Wisconsin. 109 pages, 1951. $2.75.

This book was published originally by the Academy of Science, U.S.S.R. in 1945 and comprises seven chapters: History of the Nitrogen Problem; The Forms of Nitrogen Available to Plants; Nitrogen Metabolism in Plants and the Role of Ammonia and Amides; Synthesis of Organic Nitrogenous Compounds from Nitrates and Nitrites; The Assimilation of Free Nitrogen by Plants; Relation of Plants to Ammonia and Nitrate Nutrition Depending upon the Reaction of Medium, Concentration of Solution, and Supply of Carbohydrates; and Significance of Accompanying Cations and Anions in Ammonia and Nitrate Nutrition. A bibliography of 160 references is included.

Advances in Enzymology and Related Subjects of Biochemistry, Volume XI.—Edited by F. F. Nord. Interscience Publishers, Inc., 250 Fourth Avenue, New York 1, New York. 471 pages, 1951. $9.00.

The latest volume in this series of publication includes the following articles: The Nature of Entropy and Its Role in Biochemical Processes by Herbert Gutfreund; Reaction at Interfaces in Relation to Biological Problems by J. F. Danielli and F. T. Davies; Chlorophyll Fluorescence and Photosynthesis by E. C. Wassink; Thiol Groups of Biological Importance by E. S. Guzman Barron; Pectic Enzymes by Hans Lineweaver and Eugene F. Jansen; Enzymic Synthesis of Polysaccharides, a Biological Type of Polymerization by Edward J. Hehre; The Biological Transformations of Starch by Stanley Peat; Chemical Investigations on Alliin, the Specific Principle of Garlic by Arthur Stoll and Ewald Seebeck; Some Problems of Pathological Wilting in Plants by Ernest Gäumann. Author and subject indices for this volume and a cumulative index of authors and subjects for volumes one to eleven inclusive complete the book.

Annual Review of Biochemistry, Volume XX.—Edited by J. Murray Luck. Annual Reviews, Inc., Stanford, California. 648 pages, 1951. $6.00.

This volume consists of twenty-one articles of which the following will be of special interest to plant scientists: Biological Oxidations by R. Wurmser; Nonoxidative Nonproteolytic Enzymes by W. R. Frisell and L. Hellerman; Carbohydrate Chemistry by H. O. L. Fischer and D. L. MacDonald; The Polyuronides by C. L. Hinton; Chemistry of the Lipids by K. F. Mattil; Chemistry of Amino Acids and Proteins by K. Bailey and F. Sanger; X-Ray Crystallographic Studies of Compounds of Biological Interest by R. B. Corey; Nucleic Acids, Purines, and Pyrimidines by J. Baddiley; Fat-soluble Vitamins by H. Dam; Biochemistry of Antibiotics by R. L. Peck and J. E. Lyons; Biochemical Genetics by N. H. Horowitz and H. K. Mitchell; Biochemistry of Natural Pigments (Exclusive of Haeme Pigments and Carotenoids) by T. R. Seshadri; Carbohydrate Metabolism by S. P. Colowick and N. O. Kaplan; and Water-soluble Vitamins by G. Emerson and K. Folkers. Comprehensive author and subject indices are included in the volume.

General Botany Laboratory Book.—Edward M. Palmquist and Loren C. Petry. Second Edition, 1951. W. B. Saunders Company, West Washington Square, Philadelphia 5, Pennsylvania. 190 pages, $3.00.

This book is a revised edition of the laboratory book for an introductory course in general botany first published in 1947. The new edition includes some changes suggested by the users of the book. A number of new photomicrographs are included; the treatment of mitosis and meiosis have been done over; new problems are provided on the applications of diffusion and on genetics; keys to trees have been extended to include summer conditions; and new experiments on seed germination have been added.

George W. Scarth.—The American Society of Plant Physiologists records with sorrow the passing of one of its members, George W. Scarth, Emeritus Professor of McGill University. Professor Scarth died on September 6, 1951, in Ste. Anne de Bellevue, Canada, at the age of 70.

Charles J. Engard.—Dr. Charles J. Engard, Associate Professor of Botany, Associate Plant Physiologist of the Hawaii Agricultural Experiment Station, and Chairman of the Department of Plant Physiology at the University of Hawaii, died August 22, 1951, at the age of thirty-eight. He is survived by his wife, Dorothy Doneen Engard, and two small children, Jimmy and Louise. Dr. Engard was born in Philadelphia on September 4, 1912, and received his early education in that city. He received his Bachelor of Science and Master of Science degrees from Washington State College and his Doctor of Philosophy degree from the University of Chicago in 1938. He came to the University of Hawaii in 1938 and has been associated with it since then. Dr. Engard was a vigorous, capable teacher. His extracurricular activities, most of them associated with student welfare and development, were numerous. His loss is a severe one. In research Dr. Engard's largest single contribution was reported in his book *Organogenesis in Rubus*. In it he brings from the animal morphologist the concept of field influences in determining the nature of plant organs. Still another manifestation of Dr. Engard's energetic and full life was his interest in Goethe as a biologist. He was influenced greatly by Goethe's *Morphologie* and interested Dr. Bertha Mueller, Professor of German at the University of Hawaii, to effect a translation for which he had nearly completed the introduction. This translation will soon appear from the University's press. It is always difficult for one to record the passing of a fellow scientist. It is many times more difficult when that scientist was one's own student and one who was richly endowed and who was prevented by early death from making his full contribution to the field which he loved.—Harry F. Clements.

AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS

Organized in 1924 for the advancement of Plant Physiology as a pure and applied field of botanical science

---

OFFICERS FOR 1951–52

President: Dr. Cecil H. Wadleigh,
U.S.D.A. Plant Industry Station, Beltsville, Maryland

Vice-President: Dr. Theodore C. Broyer,
University of California, Berkeley, California

Secretary: Dr. Alden S. Crafts,
University of California, Davis, California

Executive Secretary-Treasurer: Dr. J. Fisher Stanfield,
Miami University, Oxford, Ohio

Any one actively interested in Plant Physiology is eligible for membership in the Society. Candidates for membership must be recommended by two members, and approved by executive committee, or by the vote of the Society at some annual or special meeting.

The payment of the annual dues to the Society entitles the members to receive the official journal, Plant Physiology. They will receive in addition the regular bulletins and announcements sent out periodically by the secretary. Members are invited to take a personal interest in the growth and development of the Society.

Manuscripts for Plant Physiology should be addressed to Prof. Walter F. Loehwing, Department of Botany, State University of Iowa, Iowa City, Iowa. Drawings and photographs should not exceed 8.5 × 11 inches in size, should be numbered correctly on the back, and separate typewritten legends included with the text of the manuscript. Tables and cuts should be used sparingly, and the text presented in concise form. Instructions to authors for preparation of manuscripts are given in the January number, Volume 26, 1951, of Plant Physiology, pages 205–206.

CONTENTS—(*Continued*)

The Relative Herbicidal Effectiveness of Several Derivatives of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid. WENDELL R. MULLISON, Dow Chemical Company, Midland, Michigan 773

The Growth Capacity of the Sunflower Hypocotyl. R. S. DE ROSE, Lederle Laboratories, Pearl River, New York 778

Some Effects of Phosphorus on the Development, the B Vitamin Content, and the Inorganic Composition of Oats. THOMAS A. MCCOY, DAVID G. EGGERTSEN, and A. CHARLES DAVIDSON, Samuel Roberts Noble Foundation, Ardmore, Oklahoma 784

Autoradiography as an Aid in Determining the Gross Absorption and Utilization of Foliar Applied Nutrients. S. H. WITTWER and W. E. LUNDAHL, Michigan State College, East Lansing, Michigan 792

Nitrogen and Amino Acid Content of the Various Parts of Andropogon ischaemum L. GLENN S. RABINEAU and MARVIN B. EDWARDS, University of Texas, Austin, Texas 798

Occurrence of Bacillus cereus in Maryland Soils with Frenched Tobacco. ROBERT A. STEINBERG, Plant Industry Station, Beltsville, Maryland 807

Further Evidence for a Chemical Reaction between Plant Growth-Regulators and a Plant Substrate. CORWIN HANSCH, ROBERT M. MUIR, and ROBERT L. METZENBERG, JR., Pomona College, Claremont, California, and State University of Iowa, Iowa City, Iowa .. 813

The Influence of Various Sodium and Potassium Salts upon the Growth of Young Avena Seedlings. BRUCE J. COOIL, University of Hawaii, Honolulu, Hawaii 822

Brief Papers:

Ascorbic Acid and Ascorbic Acid Oxidizing Enzymes of Green Bean Plants Deficient in Manganese. KATHARINE J. HIVON, D. M. DOTY, and F. W. QUACKENBUSH, Purdue University, Lafayette, Indiana .. 832

Some Responses of Young Corn Plants to Maleic Hydrazide. LOYD A. TATUM and JOHN H. CURME, Kansas State College, Manhattan, Kansas 836

A Technique for Growing Plants under Sterile Conditions. FRANK GERMAN and VAUGHAN T. BOWEN, Brookhaven National Laboratory, Upton, Long Island, New York 840

A Technique for the Cultivation of Higher Plants under Sterile Conditions. HENRY R. KATHERINE, The Samuel Roberts Noble Foundation, Ardmore, Oklahoma 843

The Effect of Maleic Hydrazide on Tomato Plants in Relation to Their Age at the Time of Treatment. VICTOR A. GREULACH, University of North Carolina, Chapel Hill, North Carolina 848

Notes 853

General Index, Volume 20 855

THIS PUBLICATION IS REPRODUCED BY AGREEMENT WITH THE COPYRIGHT OWNER. EXTENSIVE DUPLICATION OR RESALE WITHOUT PERMISSION IS PROHIBITED.